Clinicopathologic features and genomic analysis of pulmonary blastomatoid carcinosarcoma

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Research article

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Abstract

Background: This study was designed to investigate the clinicopathologic features of pulmonary blastomatoid carcinosarcoma and explore the genomic profiles of epithelial and mesenchymal components in this tumor.

Methods: Three cases of pulmonary blastomatoid carcinosarcoma were enrolled in this study. Clinic-pathologic information and prognostic data were retrospectively reviewed. Diagnostic immunohistochemistry was performed. The epithelial and mesenchymal components were microdissected to investigate the genomic profiles by performing capture-based targeted next generation sequencing.

Results: The epithelial component in patient one was consistent of low-grade and high-grade fetal lung adenocarcinoma and displayed aberrant nuclear expression of β-catenin and missense mutation of CTNNB1 in its low-grade epithelial. The epithelial component in another two patients were consistent of high-grade fetal lung adenocarcinoma/enteric adenocarcinoma and harbored no mutation of CTNNB1. The mesenchymal components in all three tumors were primitive round/spindle cells in morphology without definite differentiation and showed cytoplasmic dot positive of β-catenin and no corresponding mutation. Within a tumor, both components exhibited relatively comparable molecular profile. In patient one, 4 mutations: RB1, FAT3, PTCH1 and LRP1B were shared by both epithelial and mesenchymal components. Epithelial had additional mutations in BCOR, CTNNB1, CTCF, FAT1 and DICER1. In patient two, 12 mutations were shared. The epithelial had BRCA2 mutations and the mesenchymal had mutations in CREBBP, ALK, DNMT3A, ASXL2, MYCN andRICTOR. Patient three had 6 shared mutations. The epithelial had an additional mutation in KAT6A and the mesenchymal had an additional mutation in APC. Collectively, we observed heterogeneity between epithelial and mesenchymal components of the same tumor.

Conclusions: Parallel detection of genetic abnormalities in epithelial and mesenchymal components of blastomatoid carcinosarcoma could provide evidence for tumor differentiation, molecular targeting and further distinguish them from conventional pulmonary blastoma and carcinosarcoma.

Introduction

The concept of blastomatoid carcinosarcoma (BCS) was initially proposed by Yukio et al in their study of classic pulmonary blastoma (CPB) and related neoplasms.[1] By definition, these tumors were categorized into one variant of carcinosarcoma with variable high-grade fetal lung adenocarcinoma (H-FLAC)/clear cell adenocarcinoma with fetal lung features and primitive mesenchymal components. Unlike CPB, both components in BCS harbored no missense mutations of the β-catenin gene. The current WHO classification suggested these entities should be classified as carcinosarcoma and the characteristic components be mentioned in pathological report. [2] However, the exact clinicopathologic features and genomic abnormalities of BCS are still poorly understood.

In clinical practice, the classification of pulmonary biphasic differentiated tumors is very difficult. These entities include classic carcinosarcoma, pulmonary blastoma, synovial sarcoma and biphasic mesothelioma. Pulmonary blastoma (PB) can be diagnosed based on typical low-grade fetal lung adenocarcinoma (L-FLAC) and unique genetic alteration involving Wnt signaling pathway which results aberrant nuclear expression of β-catenin. [3–4] Pulmonary carcinosarcoma are consisting of a mixture of non-small cell lung carcinoma and heterologous sarcomas components and can be recognized by lacking the L-FLAC component and primitive stroma of PB. [5] In addition, some gene mutations, such as TP53 mutations, commonly presented in carcinosarcoma, may assist in diagnosis. [6–7] Nevertheless, BCS seems to represent a unique subset of these tumors, not only because of its special morphology and immunophenotype, but also the complex molecular mechanism and biological behavior. At the same time, some cases may be missed or misdiagnosed due to the disunity of diagnostic criteria.

In this study, we investigated the clinicopathologic characteristics of three cases of previously diagnosed as BCS and profiled the genetic abnormalities against epithelial and mesenchymal components. The epithelial and mesenchymal components in three cases retained high consistency of genetic abnormalities in a subgroup of genes. Furthermore, the low-grade epithelial and high-grade epithelial cells showed distinctive immunophenotypes and genetic abnormalities. The mesenchymal components in three cases also displayed phenotypic heterogeneity.

Materials And Methods

1. **Patients and specimens**

Three surgically resected primary pulmonary biphasic differentiated tumors previously diagnosed as BCS were collected from the department of pathology of Shanghai Chest Hospital between May 2012 and January 2018. The resected lung specimens were taken and processed after routine internal perfusion and external fixation by 10% buffered formalin solution. Basic information of patients, grossing photographs and imaging data were reviewed from archived documents and medical records. Oncogenic driven gene mutation statuses, including *EGFR*, anaplastic lymphoma kinase (*ALK*) and *ROS1* rearrangements were routinely detected and confirmed repeatedly after surgical resection by amplification refractory mutation system (ARMS) and/or fluorescence in situ hybridization (FISH) methods. All 3 patients had wild-type *EGFR, ALK* and *ROS1*. The pathological and clinical staging were introduced according to the recommendation of the seventh edition of lung cancer. [8] Diagnosis and recognition of H-FLAC and L-FLAC were reevaluated according to the 2015 WHO classification of lung tumors by three experienced pathologists (JZ、JKZ and YCH). Our study was approved by the ethics committee (informed consent for patient biopsy) of Shanghai Chest Hospital of Shanghai Jiao Tong University. The ethics approval ID/number is KS1751. Written informed consents were obtained from all 3 patients before surgery and for publication of scientific papers in succession.

2. **Immunohistochemistry**

Immunohistochemistry was performed on 4-μm dewaxing tissue slices by using the auto-stainer GI100 (DAKO OMNIS) and automated stainer (Ventana Benchmark XT; Roche Ventana) following the manufacturer's instructions. The diagnostic primary antibodies are listed in Table 1.

3. Sequencing analysis program

a. DNA extraction and the quality assessment

For genetic analysis, hematoxylin and eosin stained sections were prepared to identify the areas of epithelial and mesenchymal components. 5~8 of 5μm unstained tissues were then obtained using laser capture microdissection in three cases. There were very fewer L-FLAC components in patient one, therefore the total DNA of epithelial components included both low-grade and high-grade FLAC. DNA was extracted from resulting tissue fragments and paired normal lung tissue. In order to guarantee the purity of the microdissected tissues, we re-stained the remaining tumor tissue to ensure that no other ingredients doped on the target tissue. Genomic DNA was extracted with the QIAamp DNA FFPE Tissue Kit (QIAGEN, Heidelberg, Germany). DNA quality was assessed by NanoDropTM 2000 (Thermo Fisher Scientific, MA, US) and agarose electrophoresis. The quantity was measured by Qubit® dsDNA HS Assay Kit on Qubit® 3.0 Fluorometer (Invitrogen, CA, US).

b. NGS library preparation

DNA shearing was performed using Covaris M220, followed by end repair, phosphorylation and adaptor ligation. Fragments of size 200–400bp were selected using Agencourt AMPure beads (Beckman Coulter, Brea, CA, USA) followed by hybridization with capture probes baits, hybrid selection with magnetic beads and PCR amplification. A bioanalyzer high-sensitivity DNA assay was performed to assess the quality and size of the fragments. 50ng of DNA was used for library construction. Twelve PCR cycles were used for library amplification. The indexed samples were sequenced on Nextseq500 sequencer (Illumina, Inc.), San Diego, CA, USA) with pair-end reads (read length 150bp).

c. Capture-based targeted sequencing data analysis

Sequencing data were mapped to the human genome (hg19) using BWA aligner 0.7.10. Local alignment optimization, variant calling and annotation were performed using GATK 3.2, MuTect, and VarScan. Variants were filtered using the VarScan fpfilter pipeline, with loci with depth less than 100 filtered out. Minimal of 5 supporting reads were needed for INDELs and 8 supporting reads were needed for SNV calling. According to the ExAC, 1000 Genomes, dbSNP, ESP6500SI-V2 database, variants with population frequency over 0.1% were grouped as SNP and excluded from further analysis. Remaining variants were annotated with ANNOVAR and SnpEff v3.6. DNA translocation analysis was performed using both Tophat2 and Factera 1.4.3.

4. Follow up

Follow-up information were retrieved from medical records center of Shanghai Chest Hospital. Patients were routinely screened by chest computed tomography (CT) every six months. The median clinical follow-up time was 68 months (range 13 to 72). The last follow-up time was February 2019.

Results

1. **Clinicopathologic features**

The patients included two males and one female, with a mean age of 54 (ranged from 38 to 78). Patient one and patient two underwent lobectomy and lymph node dissection, and the first patient received three cycles of chemotherapy subsequently. The third patient underwent three courses of neoadjuvant chemotherapy followed by surgical resection. Detailed information was summarized in Table 2. CT scans revealed that all three tumors showed well-circumscribed mass. The third tumor presented a fibrous pseudocapsule and obvious hemorrhagic necrosis due to the neoadjuvant chemotherapy. Histologically, the epithelial component in patient one consisted of most H-FLAC and fewer L-FLAC with characteristic squamoid morule structure (Figure 1). H-FLAC showed obvious nuclear atypia and mitotic activity with comedo-like necrosis. The epithelial component in patient two consisted of pure H-FLAC (Figure 2). In patient three, the epithelial was composed of labyrinth-like glands with nucleus locating at the lateral margin and the supranuclear vacuoles toward the base. Besides, some dilated and elongated glands with dirty necrosis resembling the morphology of pulmonary enteric adenocarcinoma (Figure 3). No conventional high-grade lung adenocarcinoma or other histological non-small cell lung cancer variants were found in all three tumors and the primitive mesenchymal cells showed no clear histological differentiation.

2. **Immunohistochemical findings**

The results of immunohistochemistry were summarized in Table 3 and Figure 4. Only the squamoid morule cells of the L-FLAC component but not the surrounding stromal cells in patient one showed nuclear/cytoplasmic localization of β-catenin protein. All H-FLAC and mesenchymal components showed membranous/cytoplasmic dot positive. TTF-1 was positive in epithelial components of the first two tumors. Neuroendocrine markers (CD56 and Synaptophysin) showed various degrees of expression in both epithelial and mesenchymal cells, but nuclear immunostaining for INSM1 was not detected. PanCK was entirely positive in epithelial cells while vimentin was diffusely positive in mesenchymal components. Mesenchymal markers (SMA, MyoD1) were negative in mesenchymal components. S-100 was partially expressed in mesenchymal cells of the third tumor. The proliferation index of epithelial components (30%-80%) were significantly higher than that of mesenchymal cells (15%-20%). The epithelial displaying enteric adenocarcinoma-like morphology was focally positive for CDX2 in the third patient.

3. **Genetic sequencing results**

Gene mutation spectrum was depicted in Figure 5. In three tumors, epithelial and mesenchymal retained highly coincident genetic abnormalities which involved 3 groups of genes (*FAT3/LRP1B/PTCH1/RB1 in patient one, RB1/GRIN2A/FBXW7/EGFR/LATS2/PARP4/CDK8 /SDHA/TERT in patient two* and *KRAS/BRAF/STAT3/KMT2D/CDKN2A/CDKN2B in patient three*). In patient one, tumor cells harbored missense mutation of *CTNNB1* which suggested that this mutation maintained in the small proportion of L-FLAC and was consistent with immunohistochemistry of aberrant nuclear localization of β-catenin in squamoid morule cells. *DICER1, BCOR, PTPRT, CTCF and FAT1* mutations only presented in epithelial components. In patient two, *BRCA2* mutation was found only in epithelial, while *ALK, RICTOR, IL7R, DNMT3A, ASXL2, MYCN* and *CREBBP* mutations were found only in mesenchymal component. In patient three, *KAT6A* mutation was found only in epithelial, while *APC* mutation was detected only in mesenchymal, thus the third tumor had the lowest clonal heterogeneity among three patients. Furthermore, driver mutations of *KRAS* and *BRAF* were detected in this tumor. *RB1* mutations were found in the first two patients. BCS with L-FLAC component retained mostly missense mutations while BCS with pure H-FLAC had mutations with various mutation types. TP53 germline mutation was detected in patient two and somatic *TP53* mutation was not detected in all three tumors.

Discussion

Since Yukio et al proposed the concept of blastomatoid carcinosarcoma (BCS) and described 5 cases of such lesions in 2004 [1], some primary biphasic differentiation lung tumors with blastomatoid mesenchymal had been reported in succession under this terminology. [9, 10, 11] This tumor may be confused with the traditional definition of pulmonary blastoma (PB) and carcinosarcoma, because they could have some commonalities in morphology and genomic alterations.

Pulmonary blastoma (PB), a rare subtype of sarcomatoid carcinoma with variable malignant epithelial and mesenchymal components, only accounts for < 0.1% of all resected lung cancers. [12, 13] The epithelial is essentially low-grade fetal lung adenocarcinoma (L-FLAC) and very few cases mixed with or contained pure high-grade fetal lung adenocarcinoma (H-FLAC) component. [14–15]

The major differential diagnosis of PB is carcinosarcoma and biphasic-type synovial sarcoma. The epithelial in few carcinosarcoma displayed morphology of high-grade fetal lung-like adenocarcinoma, thus interfering with accurate diagnosis. [16]

Neither the high-grade fetal adenocarcinoma or the primitive mesenchymal component is specific for diagnosis of these biphasic differentiation tumors, and even combined with comparision in the molecular level, it is extremely challenging in practical diagnosis. Previous studies demonstrate that CTNNB1 mutation and subsequent activation of the Wnt signaling pathway play an important role in tumorigenesis of L-FLAC and PB tumors [1, 3, 4]. Moreover, few cases of PB were found to harbor somatic DICER1 missense mutation and indicated that DICER1 may be closely related to these tumors presenting later in life. Our results demonstrated that aberrant nuclear expression of β-catenin and missense mutation of CTNNB1 and DICER1 were found only in L-FLAC component but not in both H-FLAC and

primitive mesenchymal cells which was further supported by previous studies [17, 18]. Therefore, we speculated the pathogenesis of H-FLAC and corresponding PB tumors are different from that of low-grade tumors, and there must be some genetic alterations that determine the pathogenesis of tumor differentiation and prognosis.

There were controversies regarding of TP53 and KRAS mutation in CPB tumors among several studies [19–21]. Somatic mutation of TP53 was not detected in any of the three patients in our study, but the third tumor was found to harbor KRAS and BRAF missense mutation. To the best of our knowledge, this is the first study reporting KRAS mutation in BCS. There are individual genetic abnormalities in each epithelial and mesenchymal component. The human FAT1 and FAT3 gene encode large proteins with extracellular Cadherin repeats that are associated with neurodevelopment and cell migration and are most homologous, which involved in tumor suppression [22]. No relevant studies or case reports have documented CPB or BSC harbored these two gene mutations. Our data indicated that FAT1 and FAT3 gene missense mutations coexisted in the epithelial and mesenchymal cells in patient 1. In addition, some genetic abnormalities, such as BRCA2 and KAT6A only occurred in H-FLAC; while FUBP1, RICTOR, and CREBBP only existed in mesenchymal components indicating the phenotypic heterogeneity among H-FLAC, L-FLAC and mesenchymal cells. These newly discovered mutations may be important evidence for the transformation of fetal adenocarcinoma from low-grade to high-grade tumor. Some powerful driver gene mutations, such as KRAS and BRAF may lead to significant morphological identification of the tumor as depicted in our case.

Single gene alteration including CTNNB1 and TP53, even combined with typical histology are insufficient to diagnose or exclude the diagnosis of PB and BCS. A small amount of L-FLAC does not affect the diagnosis for BCS. And we are of the opinion that BCS should have both a subgroup with and without L-FLAC components. The important difference between BCS and classical PB is whether there exist the mutations of CTNNB1 in both epithelial and mesenchymal cells at one tumor. As for epithelial stromal transformation, for BCS, we are more inclined to epithelial differentiation of mesenchymal components. The index suggested that epithelial cells were significantly higher than interstitial cells. And the distribution is relatively uniform. None of previously published studies extensively compared the genetic abnormalities in both mesenchymal and epithelial components of PB which contains L-FLAC and H-FLAC. Therefore, parallel detection of genetic abnormalities may provide additional information in assisting the differential diagnosis of these biphasic pulmonary tumors. Similarity of genetic alterations in epithelial and mesenchymal components can potentially reflect the origin or differentiation of the tumor, thus can be used as an important basis for diagnosis and differential diagnosis especially in biopsy or limited excision specimens.

Interestingly, the BCS tumor containing L-FLAC retained mostly genetic abnormality of missense variant while BCS with pure H-FLAC components had multiform mutation variants in our study. This may indirectly reflect that H-FLAC harbor more complex genetic abnormalities than L-FLAC and perhaps BCS with pure H-FLAC component is a separate entity from a precursor tumor.

There is still insufficient clinical evidence for standardized treatment and available recommendations for BCS and related tumors. From our clinical experience, early stage BCS tumors less than 4 centimeters without lymphatic and hematogenous metastasis could benefit from combined surgical resection and chemotherapy. Our follow-up information indicated encouraging results. However, the exact long-term behavior of is still unclear. More clinical cases, long-time follow-up information, and comprehensive genetic analysis may be helpful for establishing the behavior of BCS and the development of subsequent treatment. Thus, parallel detection of genetic alterations in this biphasic sarcomatoid carcinoma is helpful to understand the histogenesis and launch clinical studies for targeting or tolerant drugs, and provide prognostic information.

We agree that the occurrence of BCS, classical PB and carcinosarcoma are due to a group of genetic abnormalities in both epithelial and mesenchymal. Conservative or consistent genomic changes in both epithelial and mesenchymal play a decisive role in the development, and are also one of the criteria in molecular diagnosis for corresponding entities [20, 23]. At the same time, we speculate that tumors cells progress slowly with the accumulation of genetic alterations and further display these intrinsic changes in morphology and immunophenotype.

Conclusion

To the best of our knowledge, our study is the first study which extensively investigated and compared the genetic alterations of epithelial and mesenchymal components in BCS. Our research indicated that the two components retained high consistency in genetic abnormalities. we also observed heterogeneity between epithelial and mesenchymal components of the same tumor. Accurate molecular targeting detection may be significant in individualized diagnosis and treatment. The newly discovered gene mutations in combined H-FLAC and L-FLAC components, such as BCOR, PTPRT, FAT1, CTCF, may be important evidence for the transformation of epithelial from low-grade to high-grade tumor and may be related to the prognosis.

List Of Abbreviations

BCS:blastomatoid carcinosarcoma; L-FLAC:low-grade fetal lung adenocarcinoma; H-FLAC:high-grade fetal lung adenocarcinoma; CPB:classic pulmonary blastoma; ALK:anaplastic lymphoma kinase; ARMS:mplification refractory mutation system; FISH:fluorescence in situ hybridization; CT:computed tomography.

Declarations

Ethics approval and consent to participate

The Ethics Committee of Shanghai Chest Hospital of Shanghai Jiao Tong University approved this study. The patient agreed to participate in the study with all relevant data. The ethics approval ID/number is KS1751.

Consent for publication

Written informed consents were obtained from all patients before surgery and for publication of scientific papers in succession.

Availability of data and materials

The datasets including in this study are available from the corresponding author on reasonable request.

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Author contributions

YCH and JKZ designed and drafted the manuscript. JKZ and JJZ wrote the paper. JKZ, JZ and KKY completed pathological diagnosis and evaluated the results of immunohistochemistry. CX and RYZ performed the next generation sequencing and genetic analysis work. RYZ, PG and HHZ constructed the schema map and carried out data mining according to the results of genetic analysis. JKZ and KKY were responsible for immunohistochemical work and JZ guaranteed the technical support. The follow-up work and data were summarized by JZ. YCH supervised the whole project and taken responsibility for the integrity of the work. All authors have read and approved the final manuscript.

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Conflicts of interest

All authors declare that they have no competing interests.

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Tables

Antibody	Dilution	Clone	Source
panCK	1:200	AE1/AE3	ChangDao
TTF-1	1:300	SPT24	LEICA
β-catenin	1:300	β-Catenin-1	DAKO
CDX2	pre-diluted	DAK-CDX2	DAKO
CD56	1:150	123C3	DAKO
Synaptophysin	1:300	DAK-SYNAP	ZSGB-BIO
INSM1	1:200	sc-271408	SANTA CRUZ
Vimentin	1:600	V9	DAKO
α-SMA	1:300	UMAB237	ZSGB-BIO
MyoD1	1:200	EP212	ZSGB-BIO
S-100	pre-diluted	Nr.z 0311	DAKO
Ki-67	1:300	MIB-1	ZSGB-BIO

Table 1. Immunohistochemistry antibodies used for diagnosis.

Case	Gender	Age	Smoking status	Tumor location	Tumor size	Histology	Treatment	Stage	Follow up
1	Male	38	no	right lower lobe peripheral	4.0cm	mixed low-grade and high-grade fetal lung adenocarcinoma in epithelial component and primitive mesenchymal	lobectomy and lymph node dissection adjuvant three cycles of chemotherapy	T2aN0M0 IB	73 months survival
2	Male	78	50 packs/year	right upper lobe peripheral	4.2cm	pure high-grade fetal lung adenocarcinoma and primitive mesenchymal	lobectomy and lymph node dissection	T2bN0M0 IIA	68 months
3	Female	54	no	left lower lobe peripheral	3.9cm	pure high-grade fetal lung adenocarcinoma and primitive mesenchymal	chemotherapy followed by lobectomy and lymph node dissection	T2aN0M0 IB	13 months survival

Table 2. Clinicopathological characteristics of three cases of pulmonary blastomatoid carcinosarcoma in this study.

Antibody	Patient 1			Patient 2		Patient 3	
	L-FLAC	H-FLAC	Mesenchymal	H-FLAC	Mesenchymal	H-FLAC	Mesenchymal
TTF-1	+	+	-	+	-	-	-
CD56	-	focal+	-	focal+	focal+	focal+	focal+
Syn	-	-	-	focal+	focal+	-	-
INSM1	-	-	-	-	-	-	-
Vimentin	-	-	+	-	+	-	+
S-100	-	-	-	-	-	-	focal+
SMA	-	-	-	-	-	-	-
MyoD1	-	-	-	-	-	-	-
CDX2	-	-	-	-	-	focal+	-
panCK	+	+	-	+	-	+	-
Ki-67	30%	55%	15%	60%	15%	80%	20%
β-catenin	nucleus+	membrane+	dot+	membrane+	dot+	membrane+	dot+

Table 3 Immunohistochemical staining in epithelial and mesenchymal of three pulmonary blastomatoid carcinosarcoma tumors.

Figures

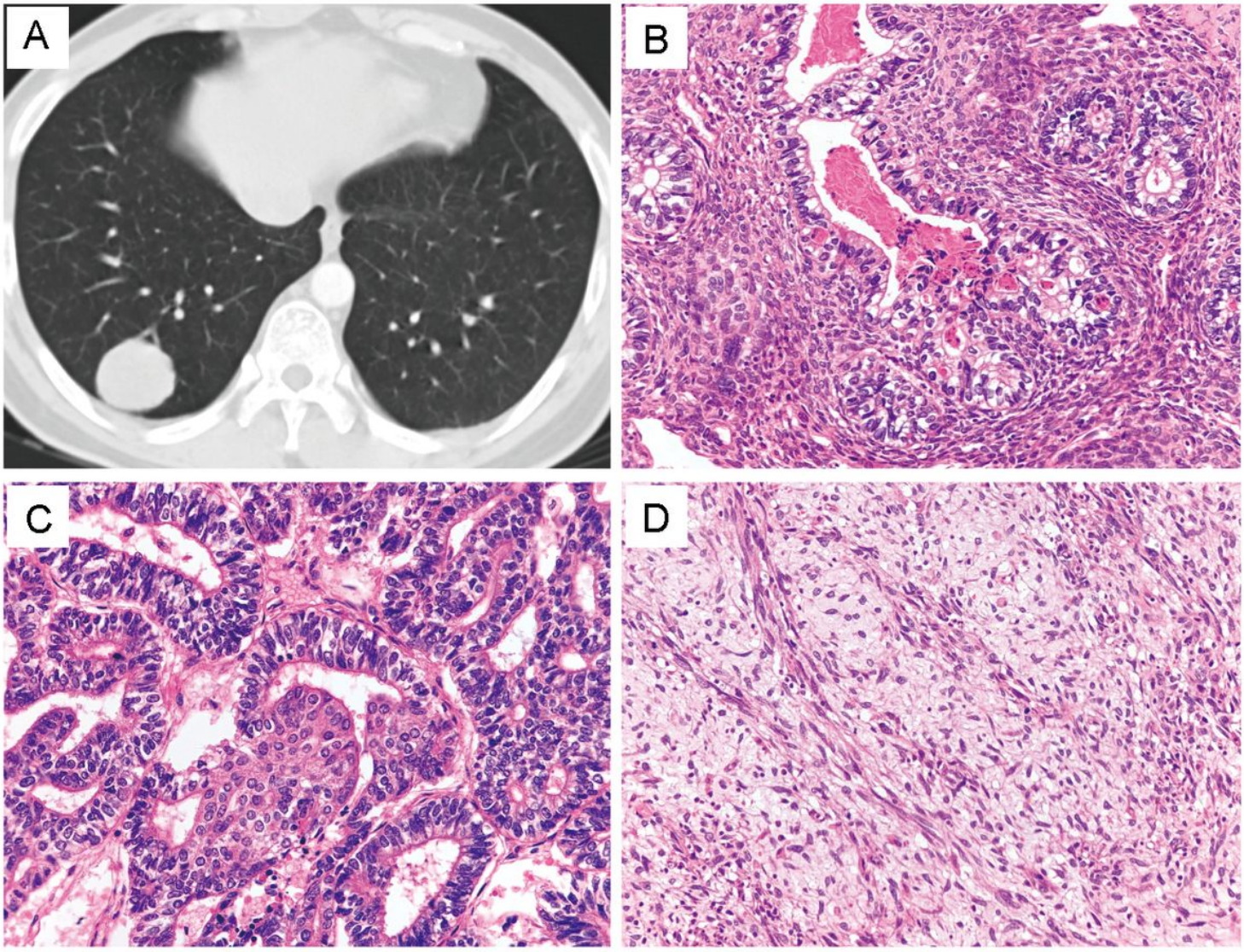

Figure 1

Imaging and morphology in patient one. (A) CT showed a soft tissue tumor in the right lower lobe. (B) The epithelial consisted of H-FLAC with marked pleomorphism and necrosis and mild L-FLAC with typical squamoid morules (C). (D) Primitive mesenchymal in some areas displayed fusiform structure and mucoid stroma.

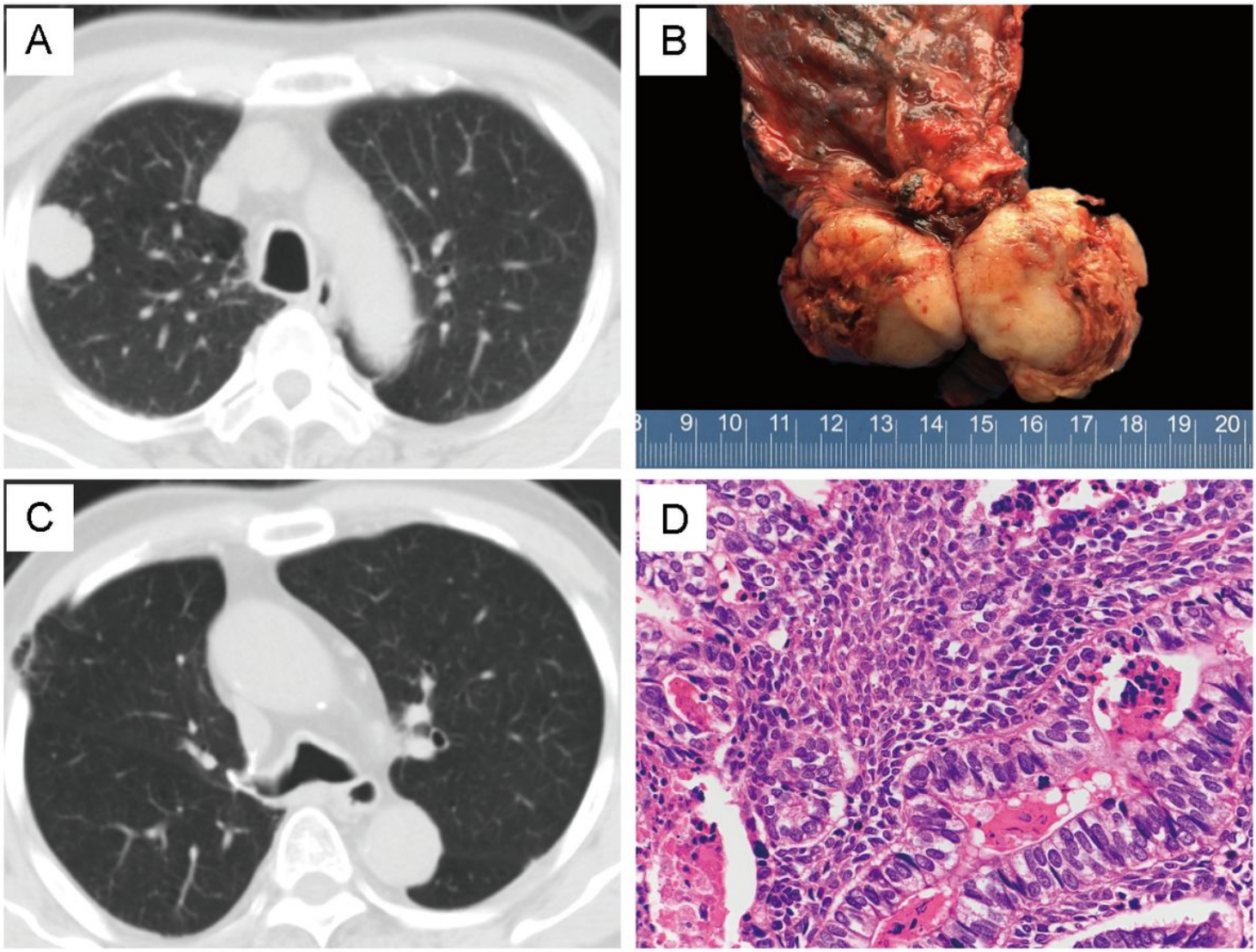

Figure 2

Imaging, grossing photograph and morphology in patient two. (A) CT displayed a peripheral mass in right upper lobe. (B) Gross appearance showed a well-circumscribed tumor with areas of necrosis and glistening homogeneous yellow-white cut surface. (C) CT revealed no signs of relapse in the primary site 48 months after surgical resection. (D) The tumor contained pure H-FLAC mixed with primitive stroma.

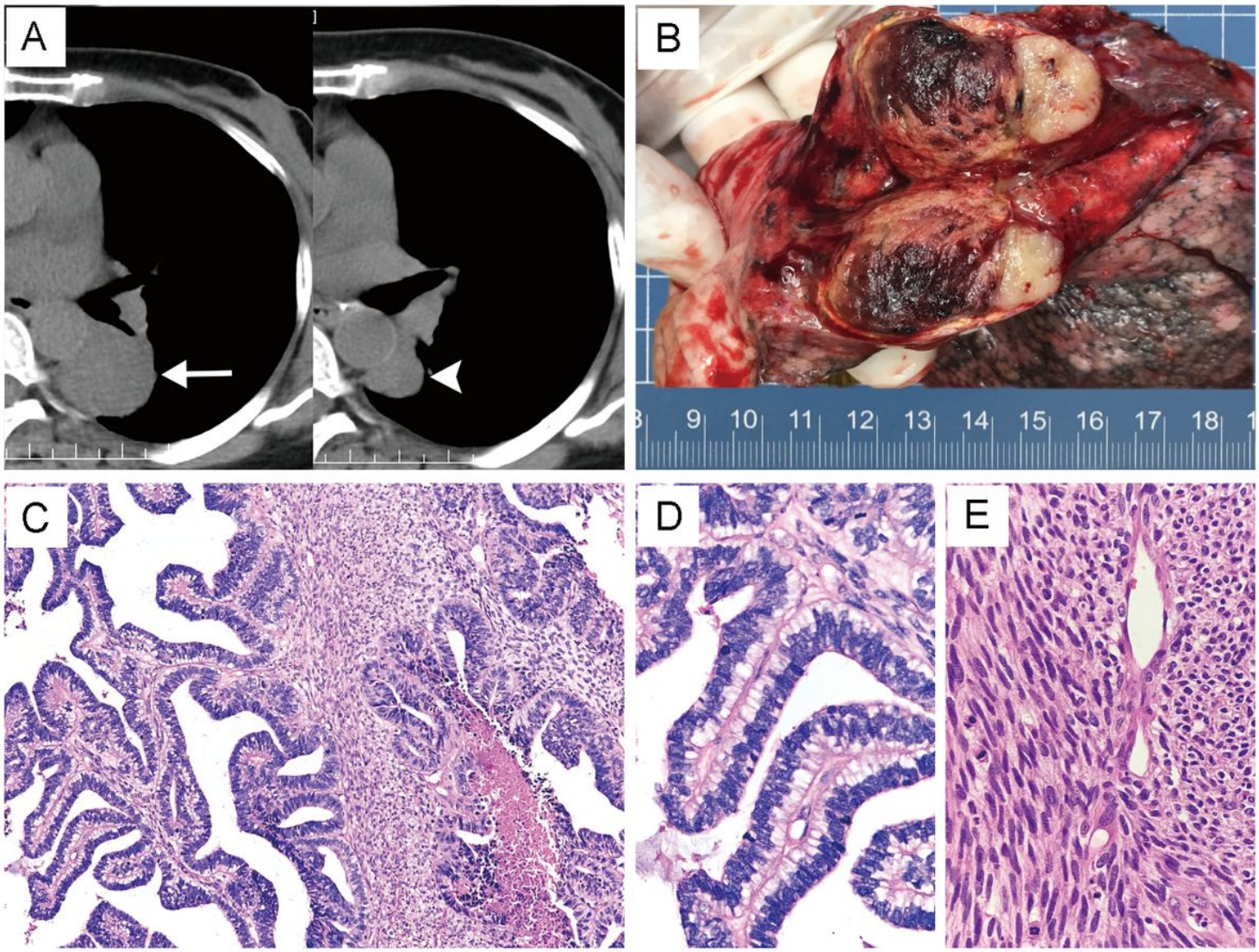

Figure 3

Imaging, grossing photograph and morphology in patient three. (A) Mediastinal window of CT indicated that the tumor shrank significantly before (white arrow) and after (arrowhead) chemotherapy. (B) Gross excision of the specimen showed obvious intratumoral hemorrhage. (C) The epithelial arranged in papillary structure and resembled the morphology of enteric adenocarcinoma at scanning magnification. (D-E) Higher magnification demonstrated the cytological features of epithelial and mesenchymal cells respectively.

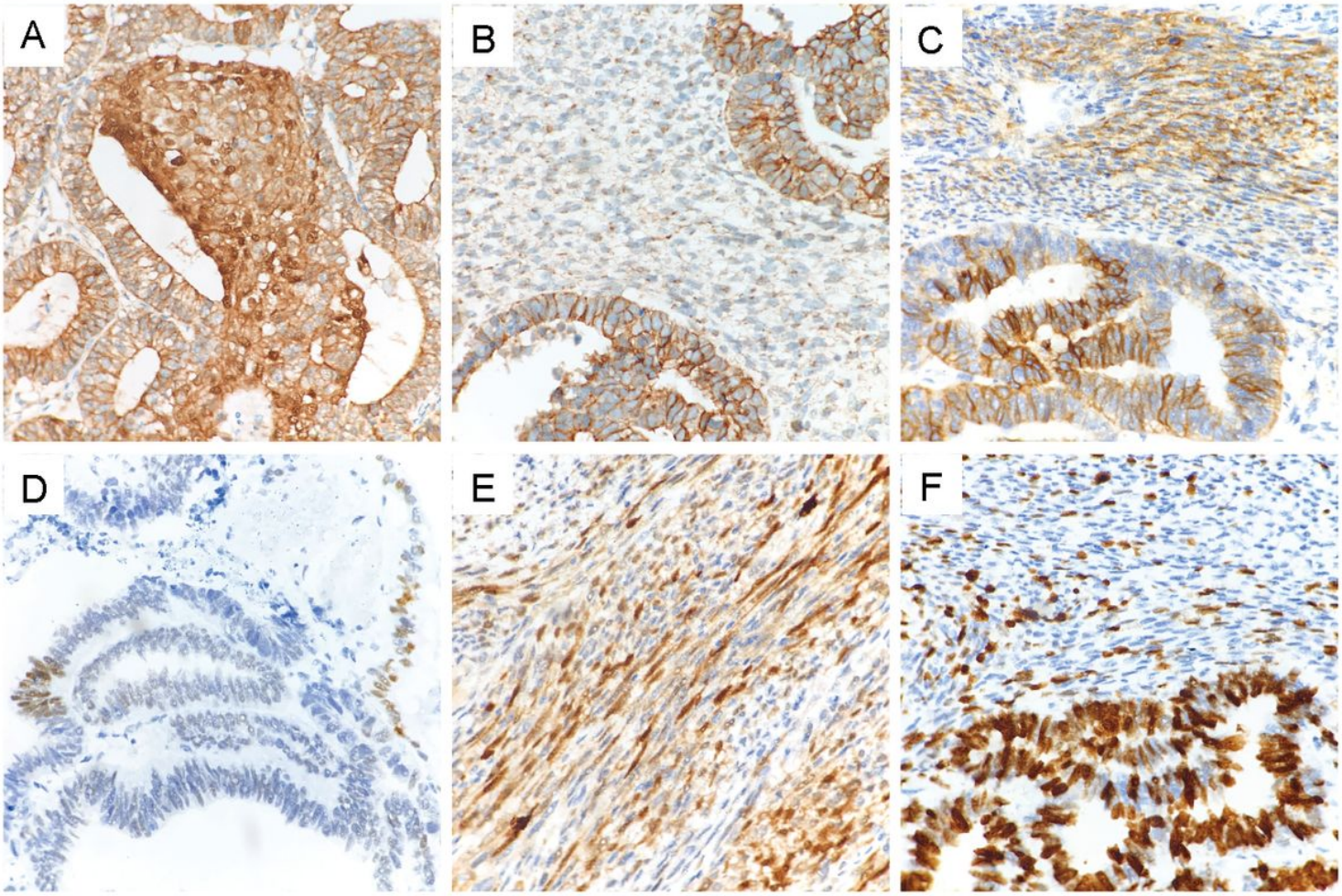

Figure 4

Immunohistochemical features of blastomatoid carcinosarcoma (BCS). (A) β-catenin was nucleus positive in squamoid morule cells of L-FLAC and membrane positive in columnar epithelial of H-FLAC and dot-positive in mesenchymal cells (B). (C) Neuroendocrine marker CD56 were expressed in both epithelial and mesenchymal cells. Some epithelial cells were positive for CDX2 (D) and the mesenchymal cells was positive for S-100 (E) in the third patient. (F) The proliferation index of epithelial components was significantly higher than that of mesenchymal.

Figure 5

Gene mutation spectrum of blastomatoid carcinosarcoma (BCS) of three cases.