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ARTICLE

Esophageal squamous cell carcinoma with basaloid features are genetically and prognostically similar to conventional squamous cell carcinoma

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We compared clinicopathologic and molecular features of esophageal squamous cell carcinoma (SCC) with basaloid features to conventional SCC using surgical resections of treatment naïve esophageal carcinomas and cases available from the TCGA database. Twenty-two cases of SCC with basaloid features were identified in the Mass General Brigham pathology archives, including 9 cases with pure basaloid morphology and 13 cases with mixed other features such as conventional well- or poorly differentiated areas or sarcomatoid areas. Thirty-eight cases of conventional SCC matched by tumor stage were used as controls. HPV infection status was tested by p16 immunohistochemistry and HPV mRNA ISH. Digital slides for 94 cases of esophageal SCC from TCGA found in the Genomic Data Commons (GDC) Data Portal were reviewed. Five cases of SCC with basaloid features were identified. Genomic profiles of SCC with basaloid features were compared to the rest of 89 SCCs without basaloid features. In addition, eight tumor sections from six patients selected from our cohort underwent in-house molecular profiling. Compared to conventional SCC, SCC with basaloid features were more frequently associated with diffuse or multifocal squamous dysplasia ($p < 0.001$). P16 IHC was positive in 2/13 cases, whereas HPV mRNA ISH was negative in 17/17 cases (including both p16-positive cases). SCC with basaloid features and conventional SCC from TCGA showed similar rates of *TP53* mutations, *CDKN2A/B* deletions, and *CCDN1* amplifications. *TP53* variants were identified in all in-house samples that had sufficient coverage. Survival analyses between SCC with basaloid features versus conventional SCC (matched for tumor stage) did not reveal any statistically significant differences. In conclusion, esophageal SCC with basaloid features has similar survival and genomic alterations to those of conventional SCC, are more frequently associated with diffuse or multifocal dysplasia, and are not associated with HPV (high-risk strains) infection.

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INTRODUCTION

Squamous cell carcinoma (SCC) is the most common type of esophageal cancer worldwide. In the United States, SCC accounts for greater than 30% of esophageal malignancies overall and over two-thirds of esophageal malignancies in Black and Asian/ Pacific Islander populations[1]. With limited Western studies to date on esophageal SCC, the rarer subtypes, including basaloid squamous cell carcinoma or SCC with basaloid features (SCC with basaloid features), remain vastly understudied.

SCC with basaloid features is a rare but distinct variant of SCC, accounting for 0.1 to 11.3% of total esophageal tumors[2–4]. SCC with basaloid features was first described in the oral cavity as an invasive cancer composed of monotonous closely packed cells with hyperchromatic nuclei, scant cytoplasm, small cystic spaces, peripheral palisading, and multinodular or solid growth patterns with foci of comedo necrosis[5,6]. Pathologically, SCC with basaloid features have been considered highly aggressive tumors due to their propensity for early lymph node involvement, vascular invasion, recurrence, and distant metastasis[7].

Clinicopathologic characterization of SCC with basaloid features in Western populations has been limited by the rarity of this disease, the paucity of studies on SCC with basaloid features outside of Asia, and the scarcity of multi-institutional studies. The existing literature features conflicting results regarding its biological behavior. Some, including the current World Health Organization Digestive System Tumors, have described esophageal SCC with basaloid features as being more aggressive and having a worse prognosis than conventional SCC[3,8–12]. Others have reported similar prognostic outcomes between SCC with basaloid features and conventional SCC[13–16].

On a molecular level, *TP53* mutations are the most frequent findings in esophageal SCC. Other genes that are commonly altered include NOTCH1(13.2%), *NFE2L2* (9.3%), *ZNF750* (9.0%), *FAT1* (8.8%), *PIK3CA* (8.2%), *EP300* (6.5%), *RB1* (4.8%) and *FBXW7* (4.2%)[17]. However, most molecular analyses did not separate SCC morphologic subtypes, and SCC with basaloid features has not been systematically evaluated.

In this study, we compared clinicopathological and molecular features and outcomes between esophageal SCC with basaloid

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Table 1. **A** Clinical characteristics of patients with SCC with basaloid features ($n = 22$) and conventional esophageal SCC ($n = 38$). **B** Histologic and Clinical Criteria of Patients with Basaloid Esophageal SCC ($n = 22$).

	SCC with Basaloid Features		Conventional SCCs
	Total $n = 22$	**Pure Basaloid $n = 9$**	
A			
Age range (mean)	53–79 (66)	58–78 (66)	47–85 (68)
Male: Female	14: 8	3: 6	19: 19
Location			
Proximal/ Mid	11	6	15
Distal	11	3	22
Size range (mean)	0.5–5.5 (2.82)	0.5–3.4 (2.14)	0.1–11.0 (3.50)
Pathologic stage of tumor (pT)			
1	13	7	19
2	4	2	4
3	5	0	14
N Stage			
N = 0	17	9	32
N = 1+	5	0	6
B			
Background esophagus			
Normal			4
Gastroesophageal reflux disease			15
Barrett's esophagus			5
Esophagitis			3
Risk Factors			
None Documented			4
Gastroesophageal reflux disease			1
Squamous dysplasia			1
Alcohol			11
Smoking			12
Immunocompromisation			3
Cirrhosis			2

features and conventional SCC using surgical resection specimens from patients with treatment naïve esophageal SCC and cases available from The Cancer Genome Atlas (TCGA) database.

MATERIALS AND METHODS

Esophageal SCC without neoadjuvant treatment resected between 1990 to 2016 were retrieved from the Brigham and Women's Hospital and Massachusetts General Hospital pathology archives. Clinical information was collected by reviewing electronic medical records. Pathologic features were evaluated by reviewing pathology reports and examining all slides for each case (by L.Z., selected cases by D.P. and V.D.). Basaloid features were defined by the presence of tumor nests with a multinodular or solid growth pattern composed of closely packed cells with enlarged, hyperchromatic nuclei that showed peripheral nuclear palisading, scant cytoplasm, with or without foci of comedo necrosis.

Four micron-thick sections of formalin-fixed, paraffin-embedded tissue were used for immunohistochemistry (IHC) and in situ hybridization (ISH), both of which were performed via standard automated techniques. Heat-induced antigen retrieval for IHC was achieved with citrate buffer (pH 6.1; Target Retrieval Solution, Dako, Carpinteria, CA) and a pressure cooker. IHC for p16 (clone ABS377, dilution 1:75; Enzo Life Sciences, Farmingdale, NY) was performed using EnVision+ horseradish peroxidase detection system (Dako). All tumors were stained for high-risk HPV using a branch chain in-situ hybridization platform (viewRNA, Affymetrix, Santa Clara, CA) with a cocktail of oligonucleotides that target the E6 and E7 transcripts and hybridize to the following high-risk HPV types: types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Hybridization was performed in an automated format (Leica Bond RX, Buffalo Grove, IL).

Digital slides for the 94 cases of esophageal SCC from TCGA found in the National Cancer Institute (NCI) Genomic Data Commons (GDC) Data Portal[18] (www.portal.gdc.cancer.gov) were manually reviewed. Five cases of SCC with basaloid features were identified: TCGA-VR-A8EX, TCGA-XP-A8T8, TCGA-IG-A625, TCGA-L5-A4OM, TCGA-IG-A3I8. These five cases were all included in the TCGA whole-exome sequencing data set on cBioPortal, which consists of 90 cases[18]. Our genetic analysis focused on the five SCC with basaloid features cases and 83 conventional SCC cases, excluding the two verrucous carcinoma cases we had previously identified[19]. We limited our analysis to genes most frequently involved in solid organ tumors[20] (Supplementary Table 1) and showed concordant alterations on cBioPortal and the GDC data portal. Alterations with discrepancies between cBioPortal and GDC data portal for each SCC with basaloid features case are listed in Supplementary Table 2. The list of gene alterations for the five TCGA SCC with basaloid features are listed in Supplementary Table 3.

Genetic alterations in selected in-house tumor tissue were analyzed by OncoPanel; a targeted massively parallel sequencing assay developed at BWH[20]. Briefly, DNA was isolated (Qiagen, Germantown, MD) after macrodissection of tumor from unstained slides with 4-micron thick sections. Library preparation was performed using 50 ng of DNA, and DNA was hybridized to custom capture probes (Agilent Technologies, Santa Clara, CA) covering 447 cancer-related genes (Supplementary Table 1). Illumina HiSeq 2500 (Illumina, San Diego, CA) was used to perform next-generation sequencing. The resulting data was analyzed with a BWH-developed bioinformatics pipeline, using a combination of publicly available and internally developed tools[20].

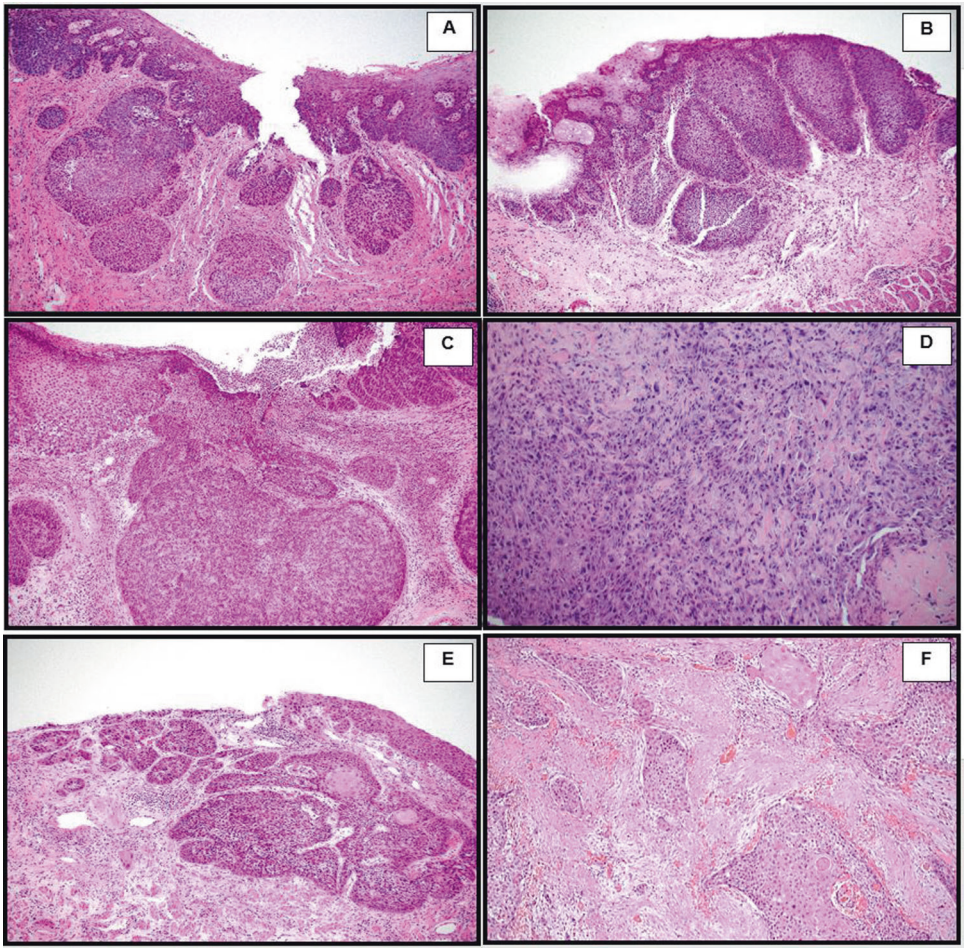

Fig. 1 Examples of esophageal squamous cell carcinoma (SCC) with basaloid features. A, **B** Examples of pure basaloid SCC. SCC with both basaloid features (**C**) and conventional poorly differentiated areas (**D**). SCC with both basaloid features (**E**) and well-differentiated keratinizing features (**F**) (**A**, **B**, **C** and **E** ×200, **D** and **F** ×400).

GraphPad Prism 8 was used for the statistical analysis. The clinicopathological data for basaloid SCC and conventional SCC groups were compared using the Fisher's exact test and the Student t-test. The 10-year survival data of patients was calculated using the Kaplan-Meier method, and the log-rank test was used to analyze the differences in survival rate between the two groups. $P < 0.05$ was considered statistically significant.

RESULTS

Clinical characteristics

Eighty-nine treatment-naïve esophageal carcinoma resections were identified, twenty-two of which demonstrated pure basaloid morphology or focal basaloid features (more than 10% of the tumor). Thirty-eight conventional SCC cases served as controls. Table 1A summarizes the clinical characteristics of our SCC with basaloid features cohort as compared to the conventional SCCs, including age, gender, tumor location, tumor size, depth of invasion, and nodal stage. Among the 22 cases of esophageal SCC with basaloid features, 9 cases showed pure basaloid morphology, and 13 cases showed additional morphologic features, including conventional well-differentiated keratinizing SCC, poorly differentiated SCC, and sarcomatoid SCC (Fig. 1).

Table 1B summarizes the background esophagus and risk factors for the SCC with basaloid features cohort. The most common risk factors included smoking (12/22, 55%) and alcohol consumption (11/22, 50%). Other reported clinical conditions included cirrhosis (2/22, 9%) or an immunocompromised state (3/22, 14%) due to a history of another cancer[1], chemotherapy[1], or stem cell transplant[1]. Four patients (18%) had no documented risk factors.

Histologic characteristics, survival and HPV status

Table 2 summarizes the histologic characteristics of the SCC with basaloid features and conventional SCC cohorts separated by tumor stage. Compared to conventional SCC, SCC with basaloid features were more frequently associated with diffuse or multifocal dysplasia (12/22, 54.5% vs. 1/37, 0.06%; $p < 0.001$). Two of the 13 cases of stage pT1 SCC with basaloid features had lymph node metastasis. But the metastatic tumors in the lymph nodes did not show any basaloid morphology. One of the metastatic tumors in the lymph node showed well-differentiated keratinizing SCC similar to what was present in the primary tumor. The other showed poorly differentiated SCC similar to its corresponding primary tumor. Among the nine cases with pure basaloid morphology, seven were pT1 tumors, and two were pT2 tumors. None of these cases had positive lymph nodes.

Ten-year survival analysis was carried out between SCC with basaloid features versus conventional SCC (matched by tumor stage) (Fig. 2). The 10-year overall survival rate was 61% for the patients with SCC with basaloid features and 50% for patients with conventional SCC. No statistical difference was seen in any of the aforementioned analyses.

P16 IHC was diffusely positive in 2 of 13 cases tested, but HPV mRNA ISH was negative in 17 out of 17 cases tested (including both cases with positive P16 IHC).

Table 2. Clinical and histologic characteristics by tumor stage.

	T1 tumors			T2 and above tumors		
	SCC with basaloid features $n = 13$	**Conventional SCC $n = 19$**	***p***	**SCC with basaloid features $n = 9$**	**Conventional SCC $n = 19$**	***p***
Age	68.5(56–79)	65.7(47–85)	0.43	66.6(53–82)	71(51–83)	0.21
Gender	Male: 7	Male: 11	0.83	Male: 7	Male: 8	0.11
	Female: 6	Female: 8		Female: 2	Female: 11	
Tumor location	Upper/mid: 8	Upper/mid: 8	0.295	Upper/mid: 3	Upper/mid:11	0.42
	Lower: 5	Lower: 11		Lower: 6	Lower: 8	
Tumor size (cm)	2.1 (0.3–3.8)	2.65 (0.5–9.5)	0.45	3.08 (1.7–5.3)	4.3 (1.9–11)	0.15
Lymph node status	Positive: 2	Positive: 0	0.038*	Positive: 4	Positive: 6	0.67
	Negative: 11	Negative:19		Negative: 5	Negative: 13	
Associated with diffuse or multifocal dysplasia	Positive: 6	Positive: 1	0.01*	Positive: 6	Positive: 0	<0.001*
	Negative: 7	Negative: 18		Negative: 3	Negative: 19	
Survival rate (80 months)	70%	80%	0.54	20%	20%	0.73

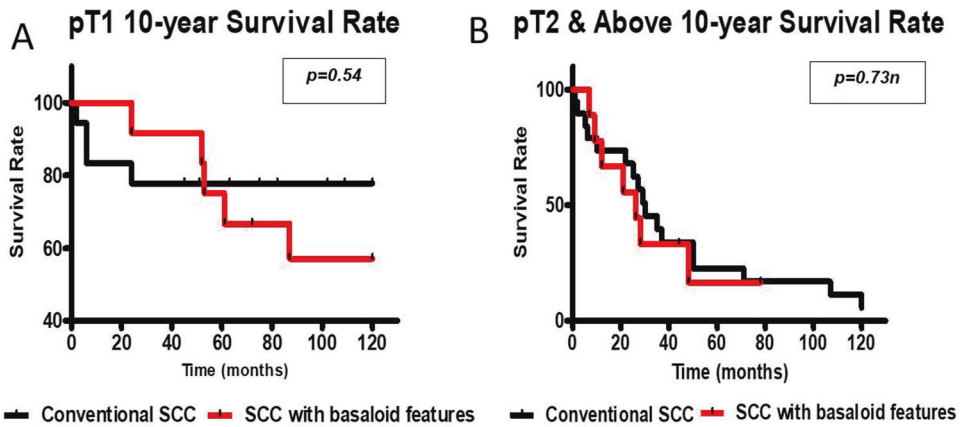

Fig. 2 Ten-year survival curves of patients diagnosed with conventional (black) and basaloid SCC (red). Survival curve (**A**) compares patients with pT1 tumors, and survival curve (**B**) compares patients with tumors pT2 and above.

Genomic characteristics

Five cases from the TCGA esophageal SCC cohort had basaloid features (Fig. 3). Esophageal SCC with basaloid features shares a similar mutational profile with conventional SCC, as summarized in Fig. 4. The most commonly mutated gene was *TP53*, mutated in 100% of basaloid SCC and 95% of conventional SCC, including missense driver and truncating mutations and one case with deep deletion. The most frequently amplified gene was *CCND1*, involving 80% of basaloid SCC and 58% of conventional SCC. *CDKN2A* was the most common gene to undergo deep deletion, affecting 80% of basaloid SCC and 58% of conventional SCC. No statistically significant genomic differences between basaloid and conventional SCC were observed in the genes analyzed.

In addition, we attempted to analyze the genomic alterations of our in-house samples. Four pure basaloid SCC and two SCC with basaloid features were selected. In the tumors with mixed morphologic components, basaloid components and conventional components were analyzed separately. A total of eight tumor blocks were sequenced in-house using OncoPanel. Although a full panel of genomic alterations was unsuccessful due to DNA decay, *TP53* variants were identified in seven of seven samples that had sufficient coverage of the *TP53* gene (Table 3).

DISCUSSION

The rarer subtypes of esophageal SCC, including basaloid squamous cell carcinoma (SCC with basaloid features), remain vastly understudied in Western populations. This multi-institutional review of 22 cases of SCC with basaloid features showed that 9 cases had pure basaloid morphology. Other cases had additional histologic features, including well-differentiated keratinizing SCC, poorly differentiated SCC, and sarcomatoid SCC. This is consistent with prior studies identifying basaloid features in conjunction with other components[7,13,21,22].

Our study also found that esophageal SCC with basaloid features is more frequently associated with diffuse or multifocal dysplasia. This is likely due to field effects related to certain risk factors such as smoking[23].

The association between HPV infection and basaloid SCC of the head and neck is well known[21,24]. However, the role of HPV in the oncogenesis of esophageal SCC remains controversial[25]. One study indicated that HPV infections occur more commonly in esophageal SCCs in Iranian populations[26]. Nevertheless, as highlighted in a recent review, integration of HPV genomes and evidence of nonintegrated HPV infection, as seen in HPV-positive SCC of the head and neck, have not been observed in esophageal SCC[27]. Furthermore, esophageal SCC rarely lack *TP53* mutations, in contrast to other HPV-positive tumors that feature E6 oncoprotein-

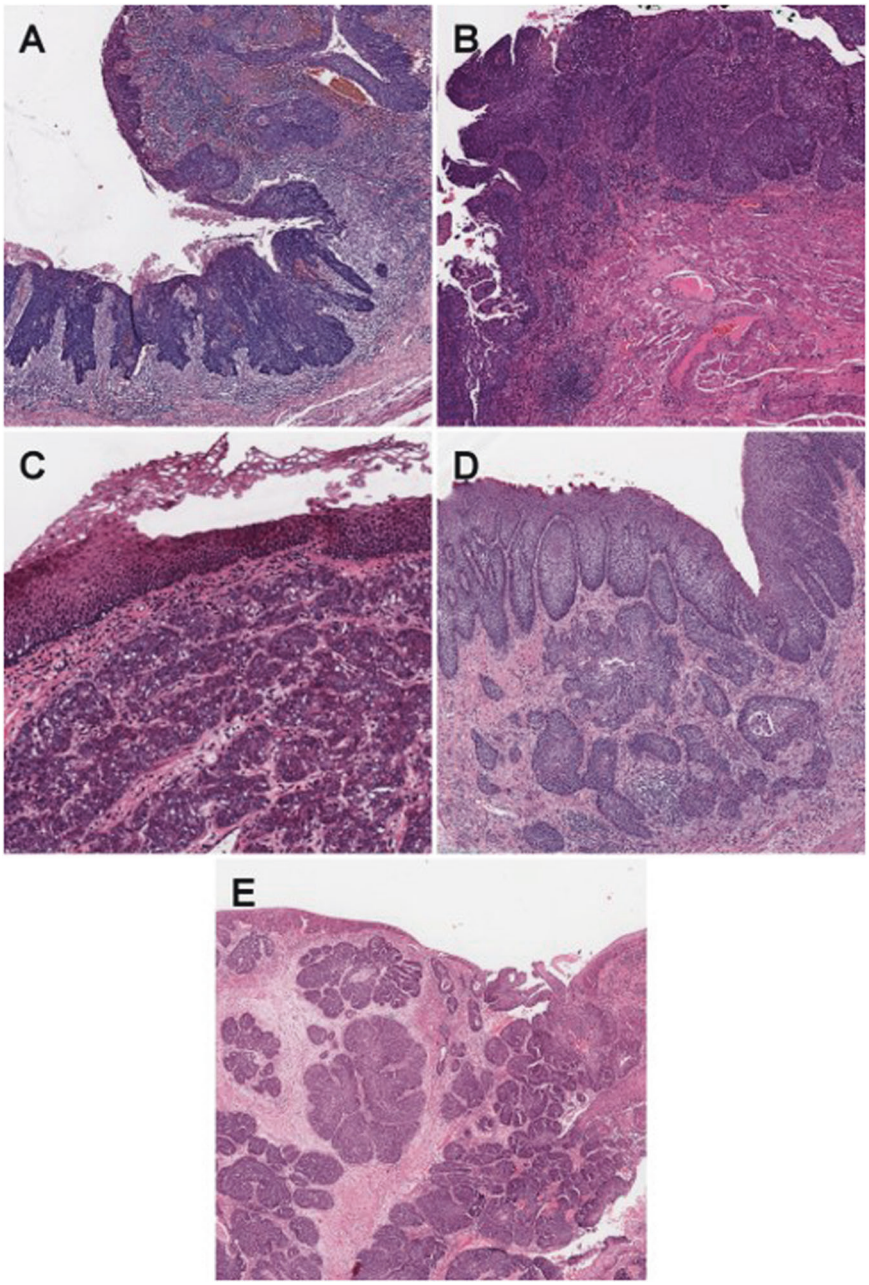

Fig. 3 Digital images of five cases of SCC with basaloid features taken from TCGA. A TCGA-VR-A8EX; (**B**) TCGA-XP-A8T8; (**C**) TCGA-IG-A625; (**D**) TCGA-L5-A4OM; (**E**) TCGA-IG-A3I8.

mediated inactivation of P53. All of the cases from our study that were tested were negative for HPV mRNA by ISH, further supporting the absence of a significant association between HPV and esophageal SCC overall and for SCC with basaloid features subtype. All of the SCC with basaloid features cases that we identified from the TCGA esophageal SCC cohort featured *TP53* mutations. We, therefore, conclude that HPV infection is an unlikely driver event in SCC with basaloid features.

Pathologically, SCC with basaloid features have been considered as highly aggressive tumors due to their propensity for early lymph node involvement, vascular invasion, recurrence, and distant metastasis[7]. We did observe lymph node involvement of two T1 tumors, but not for tumors T2 or higher when compared to stage-matched conventional SCC. Of note, the two cases showed lymph node metastasis were both SCC with basaloid features. Both showed areas of other morphology, including well-differentiated keratinizing SCC and poorly differentiated SCC. None of the tumors with pure basaloid morphology had lymph node metastasis. This suggests that basaloid morphology by itself is not prognostic of lymph node involvement.

Previous studies have indicated that SCC with basaloid features may have genomic alterations distinct from conventional SCCs and may be associated with more aggressive behavior[11,21]. Mutations in *TP53* are the most common recurrent alteration in conventional SCC, occurring in more than 90% of cases[28,29]. Our study shows that SCC with basaloid features share a similar molecular landscape with conventional SCCs, both of which have comparable rates of *TP53* mutations, *CDKN2A/B* deletions, and *CCND1* amplification. Our analyses comparing ten-year survival between SCC with basaloid features versus conventional SCC (matched by tumor stage) showed no statistically significant differences. This supports findings from prior studies reporting

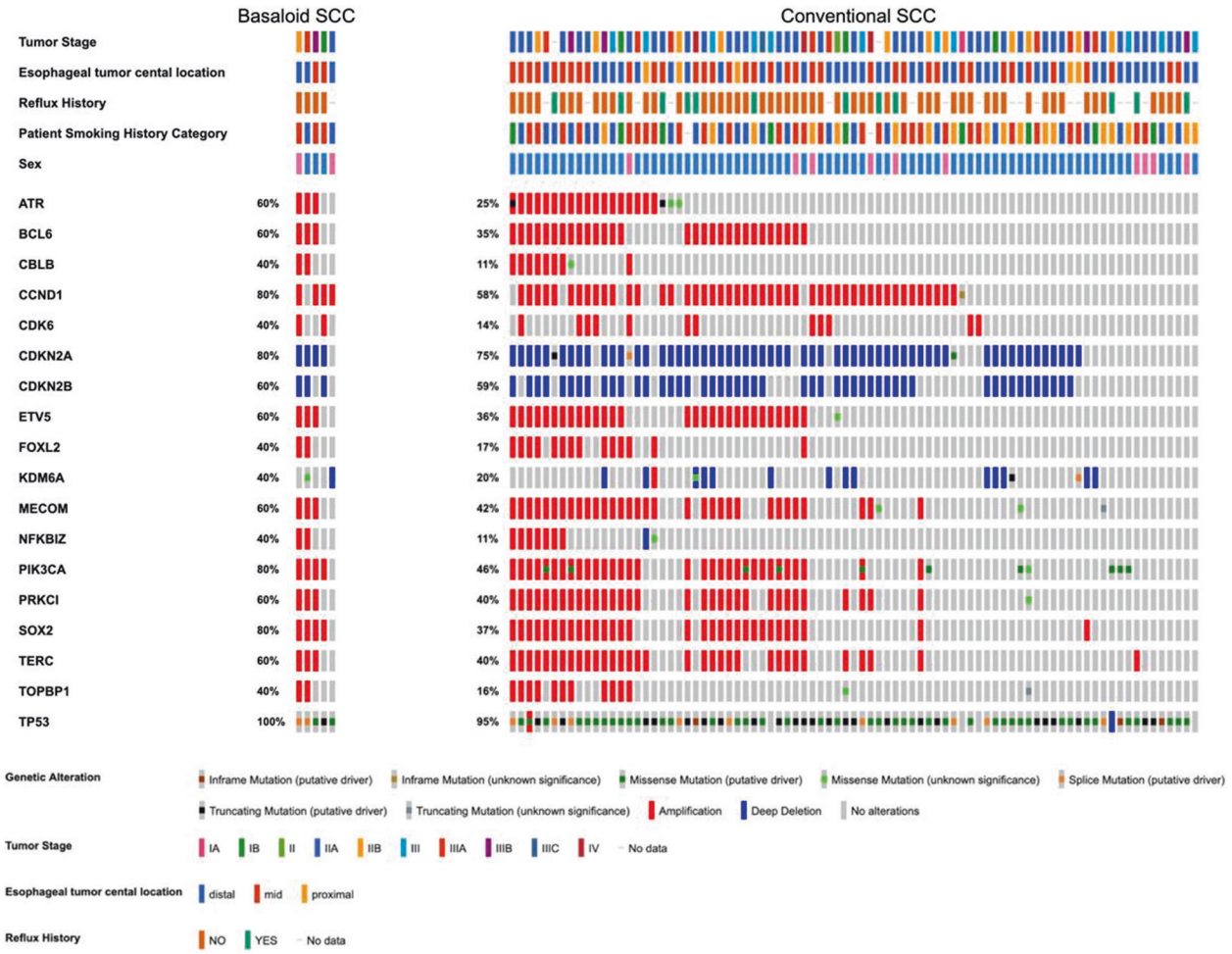

Fig. 4 Comparative genomic landscape of basaloid and conventional SCCs from TCGA. Data for genes altered in two or more cases of SCC with basaloid features are shown. Refer to Supplementary Table 3 for a complete list of cancer-related genes analyzed for the five SCC with basaloid features cases.

Table 3. Summary of molecular findings in eight tumor blocks from six patients by OncoPanel.

Patient	Gender	Histology type	Tumor purity (%)	Date of tissue collection	*TP53* variant
1	F	Pure basaloid	70	2002	p.Y220C
2	M	Pure basaloid	60	2006	p.R196* and p.G266*
3	F	Mixed, basaloid component	30	1993	p.R306*
3	F	Mixed, conventional well-differentiated component	70	1993	p.R306*
4	F	Pure basaloid	50	2016	p.R248Q
5	F	Pure basaloid	60	1991	p.R249W
6	M	Mixed, basaloid component	80	1992	FAIL
6	M	Mixed, conventional well-differentiated component	80	1992	p.H179R

similar prognostic outcomes between SCC with basaloid features and conventional SCC[14,16,30–32].

Our study has its limitations. Most notably, the number of pure basaloid SCC was small. The nine patients with pure basaloid SCC showed similar outcomes as our control group (data not shown), but the limited patient number makes the analysis less reliable. Unlike most other studies focusing on pure basaloid SCC only, we also included cases with at least 10% unequivocal basaloid component. This may make comparison with other studies challenging. However, there is an advantage of including these tumors. As a basaloid component, if present in a tumor, maybe the majority of what is seen on a biopsy specimen leading to a patient diagnosis and determining treatment. Therefore, we think the significance of an unequivocal basaloid component in SCC is also worth investigating. Our results show that pure basaloid SCC does not differ from conventional SCC on a molecular level. Furthermore, the basaloid and conventional

components within the same tumor seem to share similar tumor genomics (same *TP53* variant) (Table 3, patient 3), which confirms that basaloid differentiation is not associated with unique genetic composition.

In conclusion, our study shows that esophageal squamous cell carcinoma with basaloid features is genetically similar and does not portend a worse outcome than conventional squamous cell carcinoma. This suggests that both of these histologic subtypes may be managed similarly based on stage.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The publicly available datasets analyzed during the current study are available here: www.portal.gdc.cancer.gov.

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AUTHOR CONTRIBUTIONS

M.A.S., J.Y., and L.Z. designed the study. M.A.S., J.Y., R.A.I., F.D., A.A., D.P., J.O.W., V.D., and L.Z. collected the data. M.A.S., J.Y., R.A.I., F.D., and L.Z. analyzed the data. M.A.S. and L.Z. drafted the manuscript M.A.S., J.Y., R.A.I., F.D., A.A., D.P., J.O.W., V.D., and L.Z. revised the manuscript.

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COMPETING INTERESTS

M.A.S., J.Y., R.A.I., F.D., J.O.W., A.A., D.P., and L.Z. have no relevant financial disclosures. V.D. serves on the Scientific advisory boards of Incyte and Viela and receives research support from Advanced Cell Diagnostic and Agios.

ETHICS APPROVAL/CONSENT TO PARTICIPATE

This retrospective study was approved by the Institutional Review Board of Mass General Brigham (protocol #2018P001007). No human subjects were recruited for the study.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41379-022-01060-4.

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