Establishment and Characterization of a *TP53*-Mutated Eyelid Sebaceous Carcinoma Cell Line

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Purpose. Eyelid sebaceous carcinoma (SeC) is the third most frequent eyelid malignancy worldwide and is relatively prevalent in Asian patients. An eyelid SeC cell line model is necessary for experimental research to explore the etiology and pathogenesis of eyelid SeC. This study established and characterized an eyelid SeC cell line with a *TP53* mutation that might be useful for analyzing potential treatment options for eyelid SeC.

Methods. The eyelid SeC cell line SHNPH-SeC was obtained from a patient with eyelid SeC at Shanghai Ninth People's Hospital (SHNPH), Shanghai JiaoTong University School of Medicine. Immunofluorescence staining was employed to detect the origination and proliferation activity. Short tandem repeat (STR) profiling was performed for verification. Chromosome analysis was implemented to investigate chromosome aberrations. Whole exome sequencing (WES) was used to discover genomic mutations. Cell proliferation assays were performed to identify sensitivity to mitomycin-C (MMC) and 5-fluorouracil (5-FU).

Results. SHNPH-SeC cells were successively subcultured for more than 100 passages and demonstrated rapid proliferation and migration. Karyotype analysis revealed abundant chromosome aberrations, and WES revealed SeC-related mutations in *TP53*, *KMT2C*, and *ERBB2*. An in vivo tumor model was successfully established in NOD/SCID mice. Biomarkers of eyelid SeC, including cytokeratin 5 (CK5), epithelial membrane antigen (EMA), adipophilin, p53, and Ki-67, were detected in SHNPH-SeC cells, original tumors, and xenografts. MMC and 5-FU inhibited the proliferation and migration of SHNPH-SeC cells, and SHNPH-SeC cells presented a greater drug response than non–*TP53*-mutated SeC cells.

Conclusions. The newly established eyelid SeC cell line SHNPH-SeC demonstrates mutation in *TP53*, the most commonly mutated gene in SeC. It presents SeC properties and malignant characteristics that may facilitate the investigation of cellular behaviors and molecular mechanisms of SeC to explore promising therapeutic strategies.

Keywords: eyelid sebaceous carcinoma, *TP53* mutation, cell line

Eyelid sebaceous carcinoma (SeC) is the third most frequent eyelid malignancy worldwide and occurs more commonly in Asians, accounting for nearly 40% of all eyelid malignancies in China.[1] Eyelid SeC mainly originates from the meibomian glands, Zeis glands, and periocular skin.[2] It demonstrates regional lymph node and distant organ metastases, resulting in a 1.6% to 31.0% disease-specific mortality.[3–10] Complete excision of the tumor is the cornerstone of SeC treatment. However, some tumors present diffuse patterns that lack well-defined margins, thus making complete resection of lesions difficult. Additionally, patients with distant metastases are frequently unable to tolerate surgery.[11] Therefore, the development of effective medical treatment for eyelid SeC is warranted, particularly for eyelid SeC featuring invasive progression and poor differentiation with common mutations.

The epidemiology and etiology of eyelid SeC are poorly understood. Immunosuppression and previous radiation exposure are potential risk factors for eyelid SeC.[12] Notably, Muir–Torre syndrome, characterized by mismatch repair deficiency and microsatellite instability, is correlated with the development of extraocular SeC but exhibits an extremely rare association with ocular SeC.[13,14] With regard to genetic changes, next-generation sequencing and whole-

exome sequencing (WES) analyses have revealed that the most common mutations of SeC occur in *TP53*, accounting for 66.0% to 76.9%.[15–17] *TP53*, often described as the guardian of the genome, is essential for maintaining normal cell growth and differentiation.[18] *TP53* mutations have been discovered in various malignant tumors. These mutations prompt cells that contain damaged DNA to escape DNA repair or apoptosis and promote carcinogenesis.[19]

Establishment of eyelid SeC cell lines would provide a helpful preclinical tool to study the biology and pathogenesis of SeC, in addition to developing novel treatment options.[20] The *TP53*-mutated eyelid SeC cell line possesses the dominant molecular features of patients with eyelid SeC and serves as an ideal model for novel therapy exploration. Currently, only one SeC cell line without *TP53* mutations (BP50) has been developed,[11] but more eyelid SeC cell lines, particularly those with specific molecular characteristics, are necessary for SeC exploration.

This study aimed to establish and characterize an eyelid SeC cell line, SHNPH-SeC, derived from a primary eyelid SeC patient with a *TP53* mutation and to build eyelid SeC animal models based on SHNPH-SeC. This novel cell line may promote future research on the molecular determinants of eyelid SeC evolution and exploration of potential medical therapies.

Methods

Ethics Statement and Specimen Collection

This study followed the guidelines of the Institutional Ethics Committee of Shanghai Ninth People's Hospital, Shanghai JiaoTong University School of Medicine, in accordance with the tenets of the Declaration of Helsinki. The tumor sample for cell-line establishment was obtained from surgical resection. The tumor was initially located in the tarsus under the upper eyelid of the patient. It had gradually infiltrated the surrounding tissues and had orbital invasion. The patient underwent complete removal of the tumor with subsequent control of the disease. Written informed consent was obtained from the patient after receiving an explanation of the nature and possible consequences of the study.

Eyelid SeC Cell Line Establishment

The fresh tumor tissues were washed with Gibco PBS (Thermo Fisher Scientific, Waltham, MA, USA) and isolated from surrounding normal tissues. They were then cut into small pieces and placed into Gibco RPMI-1640 medium (Thermo Fisher Scientific), with 2% fetal bovine serum (FBS; Thermo Fisher Scientific), 200 mg/mL streptomycin (Thermo Fisher Scientific), and 200 IU/mL penicillin (Thermo Fisher Scientific). Scalpels were used to completely mince the tumor tissues, and collagenase I (Invitrogen, Waltham, MA, USA) was employed at 37°C for 4 hours. Following digestion, the tissues were centrifuged at 800 rpm at room temperature (RT) for 4 minutes. The pellet was neutralized in RPMI-1640 medium with 10% FBS, 100 mg/mL streptomycin, and 100 IU/mL penicillin after being suspended with Gibco Trypsin-EDTA (0.53-mM EDTA and 0.05% trypsin; Thermo Fisher Scientific) for 2 minutes. After another centrifugation, the cells were resuspended in fresh medium and cultured at 37°C with 5% CO_2. The cells were subcultured at a 1:3 ratio for 80 passages.

Immunofluorescence

Cells were plated onto 24-well plates with slides to make cytospins. The cytospins were fixed with 4% paraformaldehyde for 30 minutes after a 24-hour culture. They were then permeabilized in 0.5% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) for 15 minutes, blocked with 5% goat serum (Invitrogen) for 1 hour, and treated with the primary antibodies at 4°C overnight and subsequent secondary antibodies at RT for 1 hour. Nuclei counterstaining was carried out using 4′,6-diamidino-2-phenylindole (Sigma-Aldrich). An Eclipse 80i microscope (Nikon, Tokyo, Japan) was employed to acquire images. The following antibodies, all obtained from Abcam (Cambridge, UK) were used in this study: anti-cytokeratin 5 (CK5; ab52635), anti-epithelial membrane antigen (EMA; ab109185), anti-adipophilin (ab181452), anti-p53 (ab32389), and anti-Ki-67 (ab15580).

Immunohistochemistry

Tumor tissues were permeabilized in 0.1% Triton X-100 after deparaffinization and treated with 0.3% H_2O_2 to suspend endogenous peroxidase activity. The tissues were then blocked with 4% goat serum in PBS and incubated for 60 minutes with primary antibodies against CK5, EMA, adipophilin, p53, and Ki-67 at RT. We employed anti-IgG1 as the isotype control. The slides were developed using diaminobenzidine (Maxim, Shanghai, China). The slides were then examined and imaged via a Nikon ECLIPSE Ni microscope.

Cell Proliferation Assays

Cell Counting Kit-8 (CCK-8) assays were used to evaluate the capacity for cell growth. A volume of 100 µL medium and 2000 cells were added to 96-well plates. The samples were incubated at 37°C with 10 µL of CCK-8 solution (Dojindo, Kumamoto, Japan) for 2.5 hours before detection. The absorbance at 450 nm was measured using an ELX800 microplate reader (BioTek Instruments, Winooski, VT, USA).

Transwell Assays

To assess the capacity of cell migration, we employed a 24-well Transwell system with polycarbonate filters (8-µm pores). The upper compartment received a total of 5.0×10^4 cells in medium containing 2% FBS, and the lower compartment received 900 µL of complete medium. The Transwell system was dyed with 0.25% crystal violet after a 24-hour incubation. Images of the cells moving into the lower chamber were obtained.

Chromosome Analysis

Cells with 60% confluency were treated with fresh medium containing 0.2 µg/mL colchicine (Sigma-Aldrich) and incubated for 6 hours. Harvested M-phase cells were carefully resuspended in 0.075 mol/L potassium chloride hypotonic solution before being incubated at 37°C for 10 minutes. The cells were treated in a fixed solution (3:1 methanol:glacial acetic acid), extended on a slide, and stained with Invitrogen Giemsa solution. The chromosomes were visualized using a Nikon microscope and the number of chromosomes was counted and analyzed using Origin software.

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Short Tandem Repeat Analysis

Genomic DNA of tumor cells was extracted by the QIAamp PowerFecal DNA Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. Twenty-one short tandem repeats (STRs) and amelogenin loci were examined using an Applied Biosystems 3730xl DNA Analyzer (Thermo Fisher Scientific).

Whole-Exome Sequencing

A mammalian genomic DNA extraction kit (Beyotime, Shanghai, China) was used to extract genomic DNA from original tumor tissues and SHNPH-SeC cells. A NanoDrop spectrophotometer (Thermo Fisher Scientific) and 1% agarose electrophoresis were employed to determine DNA quantification and integrity, respectively. The SureSelect Human All Exon V6 library (Agilent Technologies, Santa Clara, CA, USA) was used to collect genomic DNA according to the manufacturer's instructions. The libraries were sequenced on the HiSeq X Ten sequencing platform (Illumina, San Diego, CA, USA) to generate 150-bp paired-end reads. All single nucleotide polymorphisms (SNPs), insertions and deletions (InDels), copy number variations (CNVs), and structural variations (SVs) were analyzed.

In Vivo Tumorigenesis

The xenografts were established in three 4-week-old male non-obese diabetic (NOD)/severe combined immunodeficiency (SCID) mice. The right flank of the mice was subcutaneously implanted with 1×10^6 cells. Tumor volume was evaluated every 3 days. Euthanasia was performed on the mice after 21 days. The tumors were fixed with 4% paraformaldehyde, embedded in paraffin, and stained with hematoxylin and eosin (H&E). The experiments were approved by the Animal Experimental Ethics Committee at Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine.

Statistical Analysis

The statistical analyses were performed using Prism 8.0 (GraphPad, Boston, MA, USA). The mean and SD for quantitative data are presented, and an unpaired Student's *t*-test was employed to examine the differences between the two groups. $P < 0.05$ was considered to indicate statistical significance.

Results

Biological Features of the SHNPH-SeC Cell Line

SHNPH-SeC cells showed polygonal fibroblast-like morphology, grew adherently in a monolayer, and remained stable throughout passaging (Fig. 1A). The cellular properties of SHNPH-SeC cells are consistent with the previously reported SeC cell line BP50.[11] Immunofluorescence staining demonstrated that SHNPH-SeC cells positively expressed CK5, EMA, adipophilin, p53, and Ki-67 with a 60% labeling index, which was identical to the immunohistochemical staining results of tumor tissues (Fig. 1B). These biomarkers support the notion that the SHNPH-SeC cells were derived from SeC tissues from the patient. They also confirmed that SHNPH-SeC cells expressed SeC-associated positive markers and indicated the high proliferation potential of SHNPH-SeC cells. The growth curve elucidating the doubling time of SHNPH-SeC cells was approximately 36 hours (Fig. 1C). The Transwell assay revealed that most cells migrated through the membrane within 24 hours, indicating the invasive behavior of eyelid SeC cells (Fig. 1D).

STR Authentication

STR profiling of the SHNPH-SeC cell line was performed to verify its novelty and prevent the possibility of cell-line cross-contamination. A total of 21 STRs and amelogenin loci were analyzed, and no cell lines were matched with SHNPH-SeC in the ATCC, DSMZ, JCRB, or RIKEN databases (Fig. 2A).

Chromosome Aberrations

A karyotypic study revealed numerical and structural abnormalities in SHNPH-SeC. Loss of chromosome Y and chromosomes 4, 5, 13, 15, and 21; gain of chromosome 9; and marker chromosomes of unidentified origin were the detected chromosomal numerical aberrations. Extra material of unknown derivation was discovered on chromosomes 3 and 15. A representative karyotype image is displayed in Figure 2B.

Xenograft of SHNPH-SeC

Three NOD/SCID mice that received subcutaneous injections of SHNPH-SeC cells all presented successful tumorigenesis. The final tumor volume was $467.33 \pm 51.39\,mm^3$ (Figs. 3A, 3B). H&E staining revealed SeC histological characteristics in the xenograft tumors from the mice that were consistent with the original tumor from the patient (Fig. 3C). The immunofluorescence of xenograft tumors on SeC markers indicated the same results as those of the original tumor and SHNPH-SeC cells, presenting CK5, EMA, adipophilin, p53, and Ki-67 positively (Fig. 3D).

Mutational Analysis of the Original Tumor and Derived Cell Line

WES of the original tumor and derived SHNPH-SeC cell line demonstrated their genomic mutation properties. The distribution of SNPs, InDels, CNVs, and SVs along the chromosomes of SHNPH-SeC are illustrated in Figure 4A, and the entire raw dataset is available in Supplementary Tables S1, S2, S3, and S4. A total of 22,396 SNPs of SHNPH-SeC were located mainly in the exonic region, and this number was comparable to that of the SNPs of the original tumor (24,805). SHNPH-SeC and the original tumor also share a similar composition of mutation types in the exonic region (Fig. 4B). Analysis of the mutational substitutions for the tumor and SHNPH-SeC cells exhibited coincident signatures, with A>G, C>T, G>A, and T>C being the most predominant SNP substitutions (Fig. 4C).

When comparing the SNPs and InDels of the original tumor and SHNPH-SeC cells, we identified 19,436 SNPs and 1076 InDels present in both, confirming an identical source of the tumor and SHNPH-SeC cells (Supplementary Table S5 and S6). The top 10 SeC-related genes in the COSMIC database (http://cancer.sanger.ac.uk/cosmic) and their mutation incidence are shown in Figure 4D. In both SHNPH-SeC cells and the tumor, we discovered seven shared protein-altering mutations, which were associated with the top 10 SeC-related genes (Table). Notably, *TP53*, the most frequently mutated gene in eyelid SeC, harbors a missense

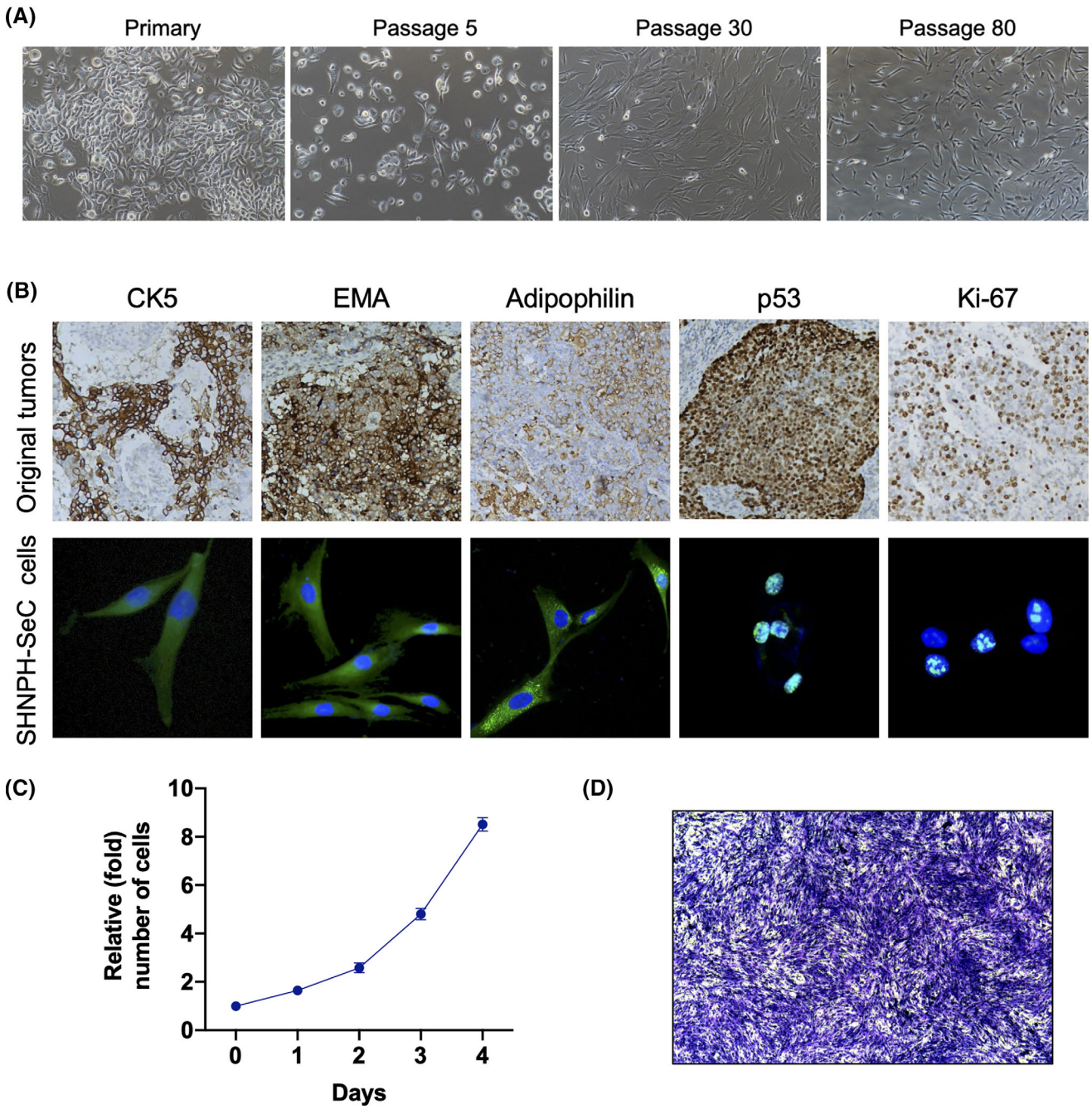

Figure 1. SHNPH-SeC cell line morphological image, immunofluorescence staining, and capacity to proliferate and metastasize. (**A**) Morphology of SHNPH-SeC cells at primary culture, passage 5, passage 30, and passage 80 (100×). (**B**) The original tumor and SHNPH-SeC cells showing positive staining for CK5, EMA, adipophilin, p53, and Ki-67 (60% labeling index; 200×). (**C**) A CCK-8 assay was employed to evaluate the proliferation of SHNPH-SeC cells. The data are presented as the mean ± SD of experimental triplicates. (**D**) A Transwell assay was employed to evaluate the migration of SHNPH-SeC cells.

mutation (c.C215G:p.P72R), and this site is known as a hotspot mutation site in malignancies.[21]

TP53-Mutated SHNPH-SeC Shows a High Sensitivity to Mitomycin-C and 5-Fluorouracil

Previous studies revealed that *TP53* polymorphisms affect individual responsiveness to cancer chemotherapy,[22,23] and patients with TP53 wild-type SeC demonstrate less sensitivity to 5-fluorouracil (5-FU), anthracycline, and cyclophosphamide than those with P72R-mutated tumors. Mitomycin-C (MMC) is a commonly used topical treatment for SeC, and 5-FU is employed for both topical treatment and systemic therapy.[12] The previously reported half-maximal effective dose (EC_{50}) of MMC in the SeC cell line BP50 without *TP53* mutations was 10.4 μM and the EC_{50} of 5-FU was approximately 80 mM.[24] We investigated the dose-dependent response in cell viability to MMC and 5-

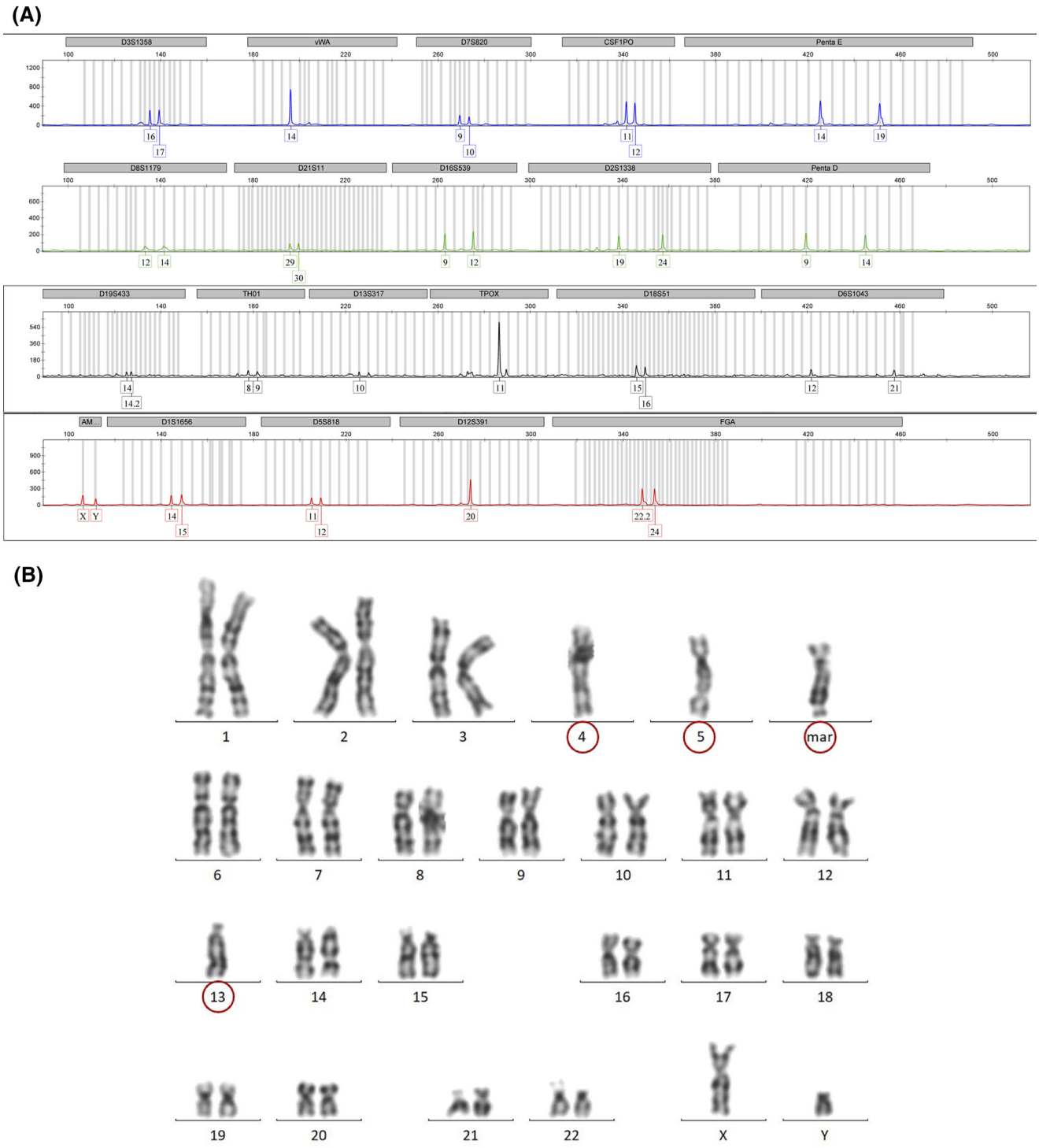

Figure 2. STR analysis and chromosome analysis of the cell line SHNPH-SeC. (**A**) An electropherogram of STR profiling showing the unique STR genotype of the SHNPH-SeC cell line. (**B**) A representative karyotype image of the SHNPH- SeC cell line, including chromosome loss of 4, 5, and 13 and chromosome gain of unidentified marker chromosomes.

FU in SHNPH-SeC cells, and the EC_{50} values of MMC and 5-FU were 1.13 µM and 83.05 µM, respectively (Figs. 5A, 5B), suggesting a higher sensitivity than BP50 cells. In addition, MMC or 5-FU treatment resulted in significant attenuation of cell growth (Fig. 5C) and migration capacity (Fig. 5D) in SHNPH-SeC cells, indicating that the established SeC cell lines are vulnerable to common chemotherapy, possibly due to *TP53* p.P72R mutation.

Discussion

Eyelid SeC is an aggressive malignancy, and metastatic eyelid SeC is life threatening.[25] Advanced SeC categorized as T4

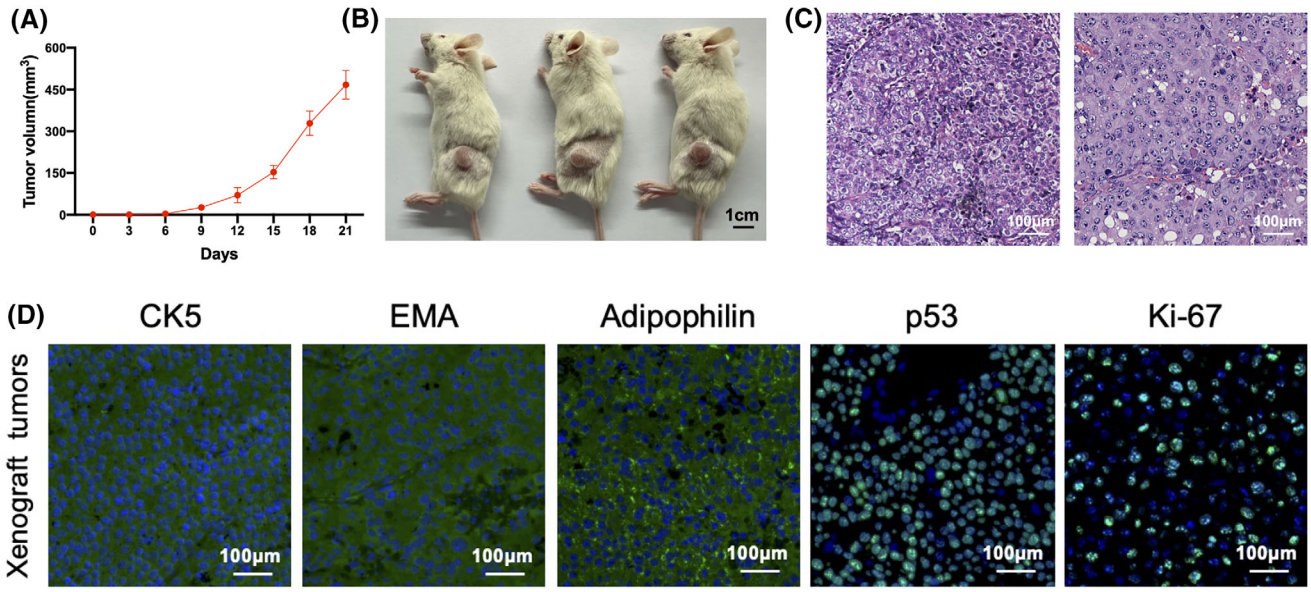

Figure 3. In vivo tumorigenicity examination and immunophenotyping of tissues. (**A**) Tumor growth curve showing the tumor volume of xerograph tumors. The data are presented as the mean ± SD of biological triplicates. (**B**) Image showing the in vivo tumors 21 days after the establishment of xenografts. (**C**) H&E staining of the original tumor and the xenografted tumor. (**D**) Xerograph tumors showing positive staining for CK5, EMA, adipophilin, p53, and Ki-67.

indicates a high risk of metastasis and poor prognosis. In this study, we established and characterized a novel SeC cell line, SHNPH-SeC, from a patient with eyelid SeC with stage T4a disease, and this cell line demonstrated a *TP53* mutation. SHNPH-SeC cells were subcultured successively for more than 100 passages, and STR analysis confirmed its novelty. The cell line was identified as a SeC cell line based on positive staining with CK5, EMA, adipophilin, and p53, which have been described as sensitive and dependable biomarkers for eyelid SeC.[26,27] The consistent staining across the original tumor, SHNPH-SeC cells, and xenograft tumors suggested that the SHNPH-SeC cells were derived from the patient with eyelid SeC. H&E staining of the original tumor and xenograft tumors further supported that SHNPH-SeC is an eyelid SeC cell line. Moreover, the identical mutation properties between the original tumor and SHNPH-SeC cells were further confirmed. Concerning malignant characteristics, the SHNPH-SeC cell line presents a high Ki-67 positive rate, various chromosome aberrations, abundant SeC-related gene mutations, a rapid proliferation and migration rate in vitro, and a capacity for tumorigenesis in vivo.

Although complete resection is the predominant treatment for SeC, it is not suitable for patients with diffuse patterns or distant metastases.[4] Our establishment of a SeC cell line with a common *TP53* mutation from invasive eyelid SeC provides an essential tool for exploring effective local and systemic medical therapy.

Genetic analysis revealed that three of the top 10 SeC-related genes share protein altering mutations in the original tumor and SHNPH-SeC cells, including those in *TP53*, *KMT2C*, and *ERBB2*. Mutations in *TP53* are a classic hallmark of cancer and trigger tumor progression in various cancers, including lung cancer, breast cancer, and liver cancer.[28–30] Multiple targeted therapies have exhibited promising results with regard to improving the survival rate for *TP53*-mutated patients.[31] For example, eprenetapopt, a small molecule restoring wild-type p53 functions in *TP53*-mutated cells, yielded high rates of clinical response and molecular remission in patients with *TP53*-mutant myelodysplastic syndrome and acute myeloid leukemia.[32] Moreover, *TP53* is the most frequently mutated gene in eyelid SeC and participates in eyelid SeC tumorigenesis,[33] suggesting that targeted therapy for *TP53* mutations may be a promising strategy for eyelid SeC treatment.

We detected the *TP53* P72R SNP (rs1042522) in SHNPH-SeC cells derived from the original tumor. It is the most common *TP53* SNP, where the nucleotide sequence CCC encoding proline (P72) is substituted by CGC encoding arginine (R72), and it is located in the proline-rich domain at codon 72.[34] Studies have revealed that *TP53* mutations containing the R72 SNP present a growth advantage, which may explain this type of cancer selection.[35] The R72 SNP also suggests a poorer prognosis than the P72 SNP in cancer.[21] Notably, the R72 SNP is reported to be more sensitive to chemotherapy than the P72 SNP. Our results verified this finding, as SHNPH-SeC cells with a *TP53* mutation (R72 SNP) manifested a better response to MMC and 5-FU than BP50 cells (P72 SNP). The evidence indicates that medical therapies may be a promising strategy, especially for *TP53*-mutated eyelid SeC patients.

KMT2C, also known as lysine methyltransferase 2C, is a regulator of H3K4 methylation and serves as a potential epigenetic driver of SeC.[15] Mutations in *KMT2C* participate in tumorigenesis by disturbing transcriptional hemostasis in multiple human cancers.[36] *ERBB2*, or erb-b2 receptor tyrosine kinase 2, is associated with the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) signaling cascade, which is identified as the frequently hijacked pathway in sebaceous carcinomagenesis.[13]

Similar to the reported SeC cell line BP50, SHNPH-SeC cells exhibit a fibroblast-like morphology. Furthermore, SHNPH-SeC cells and BP50 cells demonstrated consistent

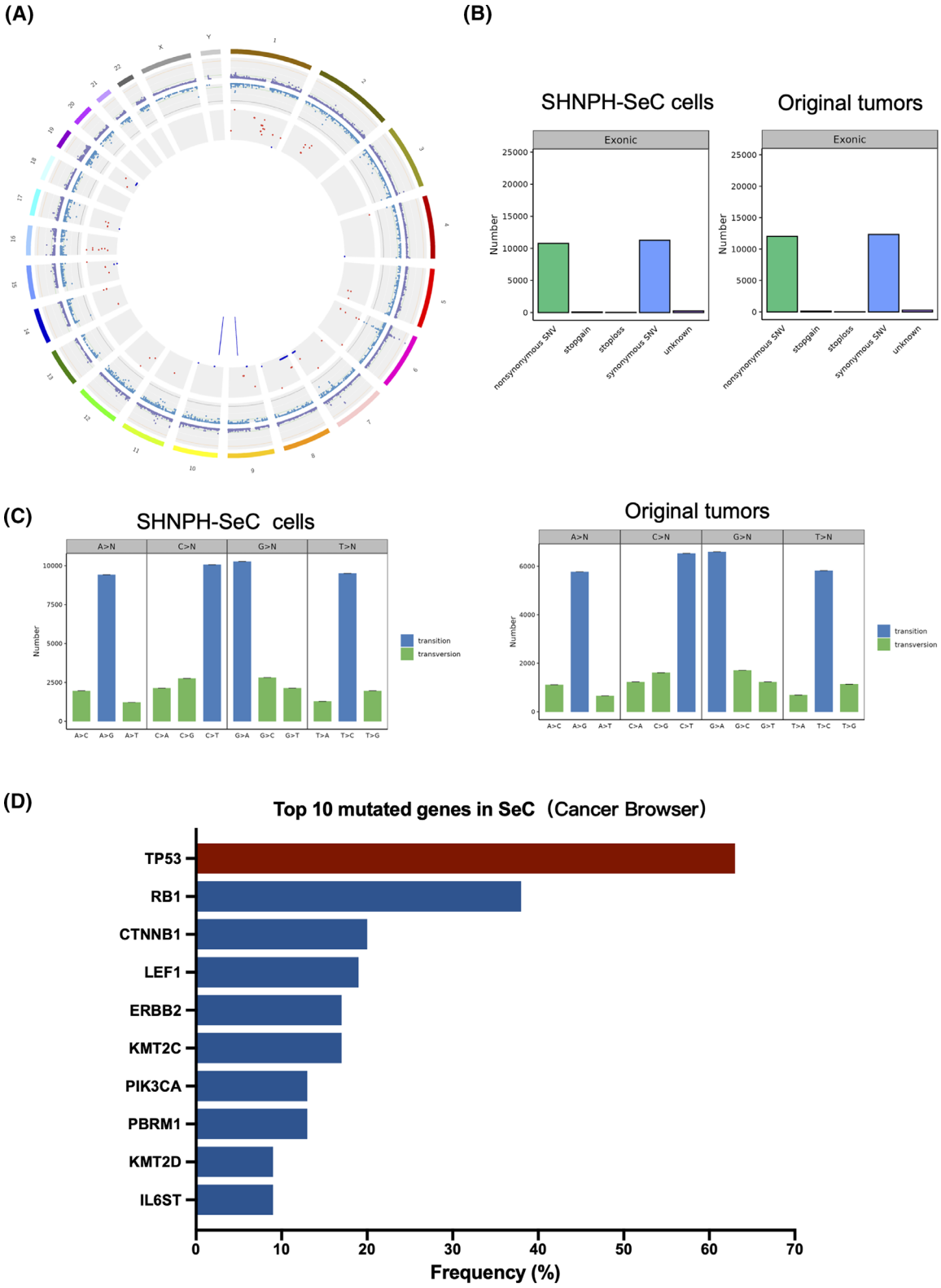

Figure 4. Mutation signatures of SHNPH-SeC. (**A**) Overview of SNPs, InDels, CNVs, and SVs in the SHNPH-SeC genome. The *outer track* shows the chromosomal ideogram. The second track demonstrates the distribution of SNP density (*purple dots*). The third track shows the distribution of InDel density (*blue dots*). The fourth track displays CNV results with copy number increase (*red dots*) and copy number deletion (*blue dots*). The fifth track shows SV results with duplication (*purple line*; support reads more than 10). (**B**) Histograms showing the number of different types of mutations in the exonic region in SHNPH-SeC cells and the original tumor. (**C**) Histograms showing the number of different base substitutions in the SHNPH-SeC cells and in the original tumor. (**D**) The top 10 SeC-related genes with their frequency are listed according to the COSMIC website.

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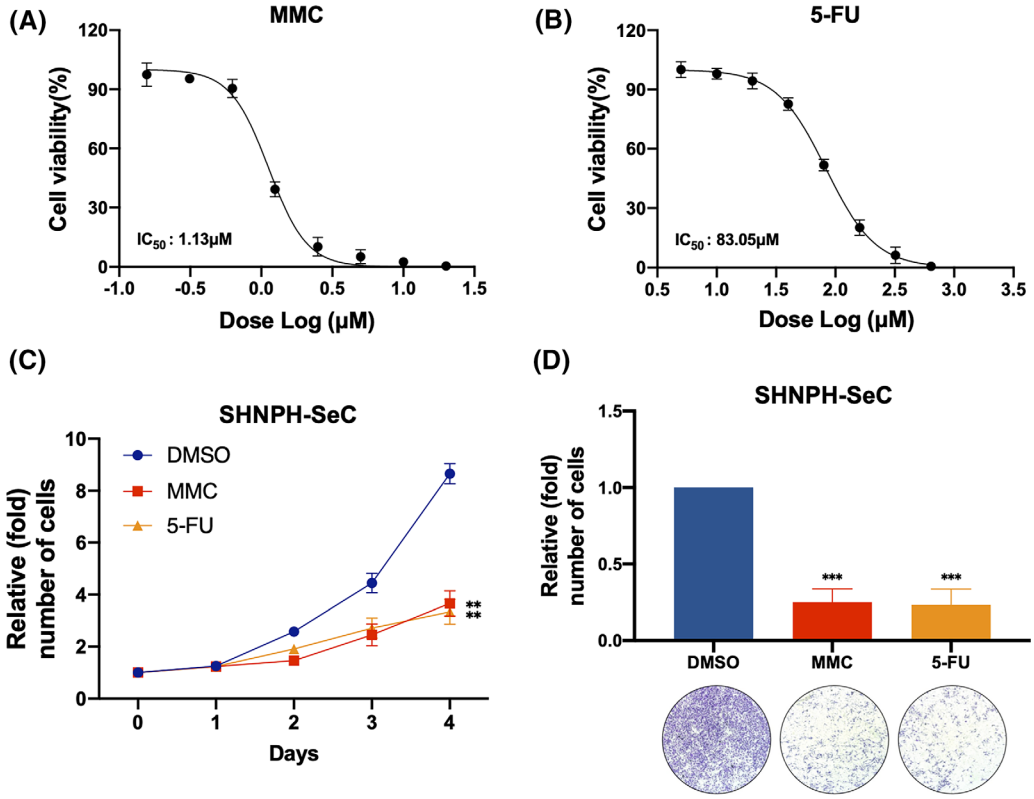

Figure 5. MMC and 5-FU inhibit the proliferation and migration of SHNPH-SeC. (**A**, **B**) Representative dose–response curves of MMC and 5-FU in SHNPH-SeC. (**C**) CCK-8 assays were employed to evaluate the proliferation of SHNPH-SeC cells upon MMC (1 μM) or 5-FU (100 μM) treatment. The data are presented as the mean ± SD of experimental triplicates. Significance was determined by unpaired two-tailed Student's *t*-test. $^{**}P < 0.01$. (**D**) A Transwell assay was employed to evaluate the migration of SHNPH-SeC upon MMC (1 μM) or 5-FU (100 μM) treatment. Representative images from three experimental replicates are shown. The data are presented as the mean ± SD of experimental triplicates. Significance was determined by unpaired two-tailed Student's *t*-test. $^{***}P < 0.001$.

Table. Shared Protein-Altering Mutations Associated With SeC in the Original Tumor and SHNPH-SeC Cells

Chromosome	Start	Ref.	Alt	Gene	Amino Acid Change
Chr7	151927021	C	A	*KMT2C*	p.C988F
Chr7	151945007	C	T	*KMT2C*	p.G838S
Chr7	151945204	G	A	*KMT2C*	p.S772L
Chr7	151970856	T	A	*KMT2C*	p.T316S
Chr7	151970931	G	A	*KMT2C*	p.L291F
Chr17	7579472	G	C	*TP53*	p.P72R
Chr17	37884037	C	G	*ERBB2*	p.P824A

expression of SeC-associated markers, such as adipophilin, EMA, and p53. However, their genetic properties are different: SHNPH-SeC cells harbor mutations in *TP53*, *KMT2C*, and *ERBB2*, and BP50 cells carry a splice-site altering variation in *RB1*, suggesting potential differences in their malignant behaviors and responses to chemotherapy.[11]

The study has some limitations. SHNPH-SeC cells and the original tumor cells varied in cellular morphology, which can potentially be ascribed to the discrepancy between the in vivo and ex vivo tumor environment. The fibroblast-like morphological transition observed in SHNPH-SeC cells, originating from the primary tumor cells, could be an adaptive response to thrive in the ex vivo setting. This could be an inevitable event during the cell line establishment, especially for the degradation of the extracellular matrix and cellular connections. The transition of fibroblast-like morphology might be attributed to the enhanced epithelial–mesenchymal transition (EMT) capacity of SeC cells, which is consistent with the increased EMT potential observed in TP53-deficient epithelial cells.[37–39] In addition, no *RB1* mutations were identified in the SHNPH-SeC cell line. *RB1* is the second most frequently mutated gene in SeC, with a mutation incidence of 38%, and it was detected in 33.3% to 68.4% of *TP53*-mutated patients in previous studies.[13,16,17,40,41] Therefore, SeC cell lines with other mutation statuses, such as the co-mutation of *TP53* and *RB1*, are necessary to establish for further exploration.

In conclusion, SHNPH-SeC is a *TP53*-mutated eyelid SeC cell line derived from an ocular SeC patient. The cell line manifests typical SeC features and malignant phenotypes. SHNPH-SeC cells responded well to chemotherapy, even more effectively than SeC cells without *TP53* mutations. Therefore, SHNPH-SeC is a useful preclinical model to explore the molecular mechanisms of eyelid SeC tumorigenesis and develop novel therapeutic options for patients with eyelid SeC.

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References

1. Shields JA, Demirci H, Marr BP, Eagle RC, Jr, Shields CL. Sebaceous carcinoma of the ocular region: a review. *Surv Ophthalmol.* 2005;50:103–122.
2. Owen JL, Kibbi N, Worley B, et al. Sebaceous carcinoma: evidence-based clinical practice guidelines. *Lancet Oncol.* 2019;20:e699–e714.
3. Kaliki S, Ayyar A, Dave TV, Ali MJ, Mishra DK, Naik MN. Sebaceous gland carcinoma of the eyelid: clinicopathological features and outcome in Asian Indians. *Eye (Lond).* 2015;29:958–963.
4. Shields JA, Demirci H, Marr BP, Eagle RC, Jr, Shields CL. Sebaceous carcinoma of the eyelids: personal experience with 60 cases. *Ophthalmology.* 2004;111:2151–2157.
5. Watanabe A, Sun MT, Pirbhai A, Ueda K, Katori N, Selva D. Sebaceous carcinoma in Japanese patients: clinical presentation, staging and outcomes. *Br J Ophthalmol.* 2013;97:1459–1463.
6. Esmaeli B, Nasser QJ, Cruz H, Fellman M, Warneke CL, Ivan D. American Joint Committee on Cancer T category for eyelid sebaceous carcinoma correlates with nodal metastasis and survival. *Ophthalmology.* 2012;119:1078–1082.
7. Sa HS, Rubin ML, Xu S, et al. Prognostic factors for local recurrence, metastasis and survival for sebaceous carcinoma of the eyelid: observations in 100 patients. *Br J Ophthalmol.* 2019;103:980–984.
8. Lam SC, Li EYM, Yuen HKL. 14-year case series of eyelid sebaceous gland carcinoma in Chinese patients and review of management. *Br J Ophthalmol.* 2018;102:1723–1727.
9. Dasgupta T, Wilson LD, Yu JB. A retrospective review of 1349 cases of sebaceous carcinoma. *Cancer.* 2009;115:158–165.
10. Gu X, Xie M, Luo Y, Song X, Xu S, Fan X. Diffuse pattern, orbital invasion, perineural invasion and Ki-67 are associated with nodal metastasis in patients with eyelid sebaceous carcinoma. *Br J Ophthalmol.* 2023;107:756–762.
11. Rong AJ, Gallo RA, Zhang MG, et al. Establishment and characterization of a novel human ocular adnexal sebaceous carcinoma cell line. *Transl Vis Sci Technol.* 2021;10:34.
12. Xu Y, Li F, Jia R, Fan X. Updates on the clinical diagnosis and management of ocular sebaceous carcinoma: a brief review of the literature. *Onco Targets Ther.* 2018;11:3713–3720.
13. Tetzlaff MT, Singh RR, Seviour EG, et al. Next-generation sequencing identifies high frequency of mutations in potentially clinically actionable genes in sebaceous carcinoma. *J Pathol.* 2016;240:84–95.
14. Singh RS, Grayson W, Redston M, et al. Site and tumor type predicts DNA mismatch repair status in cutaneous sebaceous neoplasia. *Am J Surg Pathol.* 2008;32:936–942.
15. Peterson C, Moore R, Hicks JL, et al. NGS analysis confirms common *TP53* and *RB1* mutations, and suggests *MYC* amplification in ocular adnexal sebaceous carcinomas. *Int J Mol Sci.* 2021;22:8454.
16. Tetzlaff MT, Curry JL, Ning J, et al. Distinct biological types of ocular adnexal sebaceous carcinoma: HPV-driven and virus-negative tumors arise through nonoverlapping molecular-genetic alterations. *Clin Cancer Res.* 2019;25:1280–1290.
17. Xu S, Moss TJ, Laura Rubin M, et al. Whole-exome sequencing for ocular adnexal sebaceous carcinoma suggests *PCDH15* as a novel mutation associated with metastasis. *Mod Pathol.* 2020;33:1256–1263.
18. Kiyosaki K, Nakada C, Hijiya N, et al. Analysis of p53 mutations and the expression of p53 and $p21^{WAF1/CIP1}$ protein in 15 cases of sebaceous carcinoma of the eyelid. *Invest Ophthalmol Vis Sci.* 2010;51:7–11.
19. Becker K, Goldberg M, Helmbold P, Holbach LM, Loeffler KU, Ballhausen WG. Deletions of BRCA1/2 and p53 R248W gain-of-function mutation suggest impaired homologous recombination repair in fragile histidine triad-negative sebaceous gland carcinomas. *Br J Dermatol.* 2008;159:1282–1289.
20. Akiyama T, Yoshimatsu Y, Noguchi R, et al. Establishment and characterization of NCC-GCTB5-C1: a novel cell line of giant cell tumor of bone. *Human Cell.* 2022;35:1621–1629.
21. De Souza C, Madden J, Koestler DC, et al. Effect of the p53 P72R polymorphism on mutant *TP53* allele selection in human cancer. *J Natl Cancer Inst.* 2021;113:1246–1257.
22. Bergamaschi D, Gasco M, Hiller L, et al. p53 polymorphism influences response in cancer chemotherapy via modulation of p73-dependent apoptosis. *Cancer Cell.* 2003;3:387–402.
23. Xu Y, Yao L, Ouyang T, et al. p53 codon 72 polymorphism predicts the pathologic response to neoadjuvant chemotherapy in patients with breast cancer. *Clin Cancer Res.* 2005;11:7328–7333.
24. Gallo RA, Lang SH, Gomez A, et al. Effects of mitomycin-C and 5-fluorouracil on ocular adnexal sebaceous carcinoma cells. *Am J Ophthalmol.* 2022;240:14–22.
25. Kim N, Kim JE, Choung HK, Lee MJ, Khwarg SI. Expression of Shh and Wnt signaling pathway proteins in eyelid sebaceous gland carcinoma: clinicopathologic study. *Invest Ophthalmol Vis Sci.* 2013;54:370–377.
26. Jakobiec FA, Mendoza PR. Eyelid sebaceous carcinoma: clinicopathologic and multiparametric immunohistochemical analysis that includes adipophilin. *Am J Ophthalmol.* 2014;157:186–208.e2.
27. Ansai S, Arase S, Kawana S, Kimura T. Immunohistochemical findings of sebaceous carcinoma and sebaceoma: retrieval of cytokeratin expression by a panel of anti-cytokeratin monoclonal antibodies. *J Dermatol.* 2011;38:951–958.
28. Rudin CM, Brambilla E, Faivre-Finn C, Sage J. Small-cell lung cancer. *Nat Rev Dis Primers.* 2021;7:3.
29. Shahbandi A, Nguyen HD, Jackson JG. *TP53* mutations and outcomes in breast cancer: reading beyond the headlines. *Trends Cancer.* 2020;6:98–110.
30. Bruix J, Han KH, Gores G, Llovet JM, Mazzaferro V. Liver cancer: approaching a personalized care. *J Hepatol.* 2015;62:S144–S156.
31. Anon. Glimmers of hope for targeting p53. *Cancer Discov.* 2022;12:OF5.
32. Sallman DA, DeZern AE, Garcia-Manero G, et al. Eprenetapopt (APR-246) and Azacitidine in *TP53*-mutant myelodysplastic syndromes. *J Clin Oncol.* 2021;39:1584–1594.
33. Levine AJ, Oren M. The first 30 years of p53: growing ever more complex. *Nat Rev Cancer.* 2009;9:749–758.
34. Whibley C, Pharoah PD, Hollstein M. p53 polymorphisms: cancer implications. *Nat Rev Cancer.* 2009;9:95–107.
35. Dumont P, Leu JI, Della Pietra AC, III, George DL, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. *Nat Genet.* 2003;33:357–365.
36. Lee J, Kim DH, Lee S, et al. A tumor suppressive coactivator complex of p53 containing ASC-2 and histone H3-lysine-4

methyltransferase MLL3 or its paralogue MLL4. *Proc Natl Acad Sci USA.* 2009;106:8513–8518.

37. Ohashi S, Natsuizaka M, Wong GS, et al. Epidermal growth factor receptor and mutant p53 expand an esophageal cellular subpopulation capable of epithelial-to-mesenchymal transition through ZEB transcription factors. *Cancer Res.* 2010;70:4174–4184.
38. Dong P, Karaayvaz M, Jia N, et al. Mutant p53 gain-of-function induces epithelial–mesenchymal transition through modulation of the miR-130b–ZEB1 axis. *Oncogene.* 2013;32:3286–3295.
39. Roger L, Jullien L, Gire V, Roux P. Gain of oncogenic function of p53 mutants regulates E-cadherin expression uncoupled from cell invasion in colon cancer cells. *J Cell Sci.* 2010;123:1295–1305.
40. North JP, Golovato J, Vaske CJ, et al. Cell of origin and mutation pattern define three clinically distinct classes of sebaceous carcinoma. *Nat Commun.* 2018;9:1894.
41. Bao Y, Selfridge JE, Wang J, et al. Mutations in *TP53*, *ZNF750*, and *RB1* typify ocular sebaceous carcinoma. *J Genet Genomics.* 2019;46:315–318.

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