UM-SCC-01 (FANCA<sup>+/+</sup>) UM-SCC-01ko (FANCA<sup>-/-</sup>) UM-SCC-01ko+Tg (FANCA+ transgene-complemented from clonally derived FANCA<sup>-/-</sup> subline)

#### Description

Organism: Homo sapiens Tissue: Floor of the mouth Disease: Head and neck squamous cell carcinoma Age: 73Y Gender: male HPV status: negative Synonyms: UMSCC-1; UM-SCC1; UMSCC1; SCC-1; SCC1

"UM-SCC-01" is a FANCA+/+ sporadic head and neck squamous cell carcinoma (HNSCC) cell line distributed by Millipore Sigma (SCC070). The cell line was isolated from a surgical resection of a recurrent tumor of the floor of the mouth in a 73-year-old male and established at the University of Michigan (1).

"UM-SCC-01ko" is a clonally derived FANCA-/- cell line generated from UM-SCC-01. The FANCA knockout was generated by dualgRNA cas9-mediated deletion targeting the Nterminus of the FANCA gene resulting in a loss of protein expression and function.

"UM-SCC-01ko+Tg" is a clonally derived, FANCA transgene-containing (Tg+) subline of UM-SCC-01ko in which the FANCA transgene has been inserted into the chromosome 4 'safe harbor site' 231 (SHS231) (2).

### Growth and selection media

UM-SCC-01 was grown in Dulbecco's Modified Eagle Medium – High glucose (Sigma D5796), 10% fetal bovine serum (FBS, Hyclone Laboratories SH30071.03), 1% penicillin-streptomycin (Gibco 15140122), and 1% non-essential amino acids (Gibco 11140050).

Antibiotic selection of UM-SCC-01ko+Tg cells after thawing and prior to experiments is

recommended. Selection media: 100 ug/ml hygromycin B in complete media.

## Cryopreservation and thaw protocol

Cells were cryopreserved in freezing medium containing 90% complete media and 10% DMSO.

We recommend thawing cells upon arrival to assess viability, and to do test thaws for viability when freezing liquid nitrogen stocks. Thaw the vial in a 37°C bath for 2-3 minutes before gently transferring to a centrifuge tube. Spin at 1000 RPM before resuspending in pre-warmed complete media. Plate the cells in the culture vessel of choice.

## Select genomic data

Genomic characterization of the top 12 frequently mutated genes in HNSCC (3).

Gene	TCGA mutfreq (n=276)	Mutation Status
TP53	72%	
FAT1	23%	
CDKN2A	22%	
PIK3CA	21%	
NOTCH1	19%	
KMT2D	18%	
NSD1	10%	
CASP8	9%	Nonsense
AJUBA	6%	
FBXW7	5%	
HRAS	4%	
PTEN	2%	

Additional genomic characterization can be found in the resource manuscript (4) and following publications (5,6).

# **Quality Control Testing**

The terminal expansion samples of the complete trio set were sent to IDEXX BioAnalytics (Columbia, MO, USA) for STR authentication using their Cell Check 9 Plus service. Cells were simultaneously tested for *Mycoplasma* and interspecies contamination from mouse, rat, African green monkey, and Chinese hamster. Distributed cell lines all had unambiguous

STR profile data and were negative for all screened contaminants.

- A Human 9 species-specific STR marker profile was established for the cell line pair. The unique marker results from our recent analysis are listed in STR profiling panels below.
- Test thaws indicate ≥ 50% survival of cells.

STR: ATCC	STR: IDEXX	STR: IDEXX	STR: IDEXX
UM-SCC-01	UM-SCC-01	UM-SCC-01ko	UM-SCC-01ko+Tg
AMEL: X, Y	AMEL: X	AMEL: X	AMEL: X
CSF1PO: 10,12	CSF1PO: 10,12	CSF1PO: 10,12	CSF1PO: 10,12
D13S317: 8,11	D13S317: 8,11	D13S317: 8,11	D13S317: 8,11
D16S539: 12,13	D16S539: 12,13	D16S539: 12,13	D16S539: 12,13
D5S818: 10,13	D5S818: 10,13	D5S818: 10,13	D5S818: 10,13
D7S820: 9,12	D7S820: 9,12	D7S820: 9,12	D7S820: 9,12
TH01: 6	TH01: 6	TH01: 6	TH01: 6
TPOX: 8,11	TPOX: 8,11	TPOX: 8,11	TPOX: 8,11
vWA: 15,18	vWA: 15,18	vWA: 15,18	vWA: 15,18

Note: Previous reports have identified differences in the Amelogenin marker for UM-SCC-01 that are dependent on the passage number (7). The UM-SCC-01 parent cell line included in the FA-CCLR matches the reported STR profile for the long-term passage line. **More extensive STR profiling data can be found the following publications (8,9).** 

## General workflow



**Figure 1**: Workflow for generating, characterizing, and complementing *FANCA* isogenic cell line trios starting from sporadic HNSCC cell lines. (A) Generation of *FANCA* clonal knockouts. (B) Complementation of *FANCA* clonal knockouts.

# The Fanconi Anemia Cancer Cell Line Resource

#### Verifying FANCA KO and FANCA complemented clones

The following molecular, biochemical, and phenotypic assays were used to verify the generated *FANCA* knockout and *FANCA* complemented clone. A detailed description of the protocols to generate isogenic pairs and trios and results will be included in the manuscript.



**Figure 2:** Verification of *FANCA* KO and *FANCA* complemented clones. (A, B) Representative PCR results for detection of (A) wild type FANCA and dual gRNA/cas9 mediated FANCA ko alleles and (B) SH231 site specific insertion of FANCA complementation vector, pSH231-EF1-FANCA-Hygro. (C) Representative immunoblot result for detection of FANCA protein in 30 µg of whole cell lysates. (D) Assessment of cell survival by alamarBlue assay 4 days after continuous exposure to mitomycin C (MMC).

### Protocols

### 1. Molecular detection of FANCA wild type (WT) and knockout (KO) alleles:

Primers used:

- Forward primer sequence: 5' AATTGTTCTCCCGTCTGCTCTC 3'
- Reverse primer sequence: 5' GGGCCGTCTCCGTTAGTTTC 3'

#### PCR conditions:

- Denaturation: 94°C, 20 seconds
- Annealing: 56°C, 20 seconds
- Extension: 72°C, 2 minutes
- Number of cycles: 35

Results: The two predicted PCR products using these primer sequences include a roughly 545 base pair deletion band (FANCA KO) and 1979 base pair intact band (FANCA WT). Both UM-SCC-01ko and UM-SCC-01ko+Tg cells will show the roughly 545 base pair deletion band, while the UM-SCC-01 cells will show the 1979 base pair wild-type intact band.

# 2. For detection of FANCA complementation vector in safe harbor site 231 (SH231):

Primers used:

- Forward primer sequence: 5' AGAACATGCAATGGCTAGC 3'
- Reverse primer sequence: 5' GCGGTGGTTGACCAGACAAA 3'

Results: The *FANCA* complemented, UM-SCC-01ko+Tg cells, display the predicted 634 base pair products, which represents of site-specific orientation.

## 3. Western blot of FANCA protein

Antibody used:

- FANCA primary antibody: Rabbit polyclonal (Bethyl laboratories #A301-980A)
- Secondary antibody: Goat anti-rabbit IgG, HRP conjugated (InVitrogen #G21234)

Results: UM-SCC-01 cells and FANCA complemented UM-SCC-01ko+Tg have a band indicating the presence of full-length wild type FANCA, whereas in UM-SCC-01ko cells have no detection of the full-length wild type FANCA protein due to the deletion in the *FANCA* gene.

## 4. Phenotypic analysis using a Mitomycin C sensitivity assay

Reagents:

- Mitomycin C (Millipore Sigma Cat. #M4287)
- alamarBlue Cell Viability Reagent (ThermoFischer Scientific DAL1025)

Procedure:

- 1. 2,500 cells are seeded per well in 48-well plates.
- 2. Cells are treated with varying concentrations of MMC ranging from 0nM to 80nM in fresh culture medium.
- 3. Incubate for 4 days.
- 4. Determine relative cell viability using the alamarBlue Cell Viability Reagent.

Results: UM-SCC-01ko demonstrate distinct hypersensitivity to MMC, while UM-SCC-01ko+Tg will show restored resistance comparable to wild type UM-SCC-01 cells.

### References

- Krause, C. J., Carey, T. E., Ott, R. W., Hurbis, C., McClatchey, K. D., & Regezi, J. A. (1981). Human squamous cell carcinoma. Establishment and characterization of new permanent cell lines. *Archives* of otolaryngology (Chicago, III. : 1960), 107(11), 703–710. https://doi.org/10.1001/archotol.1981.00790470051012
- Pellenz, S., Phelps, M., Tang, W., Hovde, B. T., Sinit, R. B., Fu, W., Li, H., Chen, E., & Monnat, R. J., Jr (2019). New Human Chromosomal Sites with "Safe Harbor" Potential for Targeted Transgene Insertion. *Human gene therapy*, *30*(7), 814–828. <u>https://doi.org/10.1089/hum.2018.169</u>
- 3. Cancer Genome Atlas Network (2015). Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature*, *517*(7536), 576–582. <u>https://doi.org/10.1038/nature14129</u>
- 4. Tang, W. et al. (2022). Fanconi anemia-isogenic head and neck cancer cell lines a resource for basic and translational science (in preparation for submission Q1 2022. Watch BiorXiv for pre-print)
- Cheng, H., Yang, X., Si, H., Saleh, A. D., Xiao, W., Coupar, J., Gollin, S. M., Ferris, R. L., Issaeva, N., Yarbrough, W. G., Prince, M. E., Carey, T. E., Van Waes, C., & Chen, Z. (2018). Genomic and Transcriptomic Characterization Links Cell Lines with Aggressive Head and Neck Cancers. *Cell reports*, 25(5), 1332–1345.e5. https://doi.org/10.1016/j.celrep.2018.10.007
- Kalu, N. N., Mazumdar, T., Peng, S., Shen, L., Sambandam, V., Rao, X., Xi, Y., Li, L., Qi, Y., Gleber-Netto, F. O., Patel, A., Wang, J., Frederick, M. J., Myers, J. N., Pickering, C. R., & Johnson, F. M. (2017). Genomic characterization of human papillomavirus-positive and -negative human squamous cell cancer cell lines. *Oncotarget*, 8(49), 86369–86383. <u>https://doi.org/10.18632/oncotarget.21174</u>
- Brenner, J. C., Graham, M. P., Kumar, B., Saunders, L. M., Kupfer, R., Lyons, R. H., Bradford, C. R., & Carey, T. E. (2010). Genotyping of 73 UM-SCC head and neck squamous cell carcinoma cell lines. *Head & neck*, 32(4), 417–426. https://doi.org/10.1002/hed.21198
- Zhao, M., Sano, D., Pickering, C. R., Jasser, S. A., Henderson, Y. C., Clayman, G. L., Sturgis, E. M., Ow, T. J., Lotan, R., Carey, T. E., Sacks, P. G., Grandis, J. R., Sidransky, D., Heldin, N. E., & Myers, J. N. (2011). Assembly and initial characterization of a panel of 85 genomically validated cell lines from diverse head and neck tumor sites. *Clinical cancer research: an official journal of the American Association for Cancer Research*, *17*(23), 7248–7264. <u>https://doi.org/10.1158/1078-0432.CCR-11-0690
  </u>
- Yu, M., Selvaraj, S. K., Liang-Chu, M. M., Aghajani, S., Busse, M., Yuan, J., Lee, G., Peale, F., Klijn, C., Bourgon, R., Kaminker, J. S., & Neve, R. M. (2015). A resource for cell line authentication, annotation and quality control. *Nature*, *520*(7547), 307–311. <u>https://doi.org/10.1038/nature14397</u>

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#### Datasheet version: 03172025