CCH-SCC-FA1 (*FANCA*$^{-/-}$ FA-patient derived line)
CCH-SCC-FA1+Tg(LV) (*FANCA*+ transgene-complemented)

Description

Organism: *Homo sapiens*
Tissue: Tongue
Disease: Head and neck squamous cell carcinoma
Age: N/A
Gender: male
HPV status: negative
Synonyms: CCH-FAHNSCC-2

"CCH-SCC-FA1" is a head and neck squamous cell carcinoma (HNSCC) cell line derived from a primary tongue lesion from a *FANCA*$^{-/-}$ Fanconi Anemia patient. Additional details regarding CCH-SCC-FA1 can be found in the resource manuscript (1).

"CCH-SCC-FA1+Tg(LV)" is the transgene-containing (Tg+) subline of CCH-SCC-FA1 in which the FANCA transgene was inserted via lentiviral complementation. This is a pooled cell line composed of ≥ 200 clones. Additional details regarding the lentiviral vector can be found in the resource manuscript.

Growth and selection media

CCH-SCC-FA1 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 CCH-SCC-FA1+Tg(LV) cells after thawing and prior to experiments is recommended. Selection media: 1 ug/ml Puromycin 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 (2).

Gene	TCGA mutfreq (n=276)	Mutation Status
TP53	72%	CNV Del
FAT1	23%	CNV Del
CDKN2A	22%	
PIK3CA	21%	CNV Amp
NOTCH1	19%	CNV Del
KMT2D	18%	
NSD1	10%	Multi-SNV/Indel
CASP8	9%	
AJUBA	6%	
FBXW7	5%	
HRAS	4%	
PTEN	2%	

Additional genomic characterization can be found in the resource manuscript and following publications (3-5).

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. There were no previous published STR profile for CCH-SCC-FA1 for comparative analysis. The unique marker results from our recent analysis are listed in STR profiling panels below.
- Test thaws indicate ≥ 50% survival of cells.

STR: IDEXX	STR: IDEXX
CCH-SCC-FA1	**CCH-SCC-FA1+Tg(LV)**
AMEL: X, Y	AMEL: X, Y
CSF1PO: 10	CSF1PO: 10
D13S317: 12	D13S317: 12
D16S539: 10, 11	D16S539: 10, 11
D5S818: 11	D5S818: 11
D7S820: 10	D7S820: 10
TH01: 7, 8	TH01: 7, 8
TPOX: 8,11	TPOX: 8,11
vWA: 16	vWA: 16

IDEXX STR Profile ID: IBA# 134036-22-19

General workflow

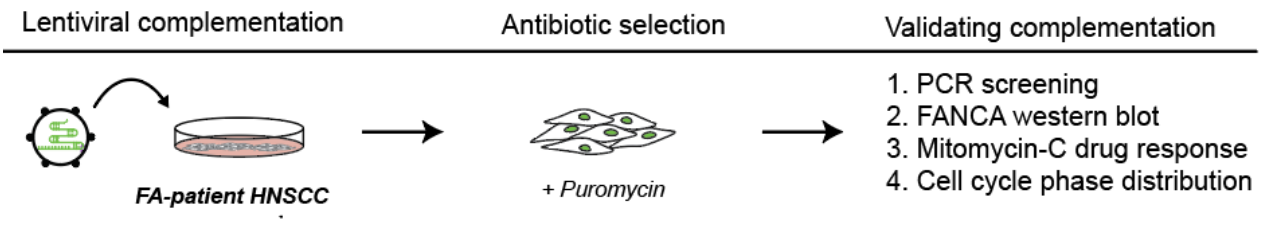

Figure 1: Workflow for generating, characterizing, and complementing *FANCA* isogenic cell line pairs starting from FA-patient derived HNSCC cell lines.

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

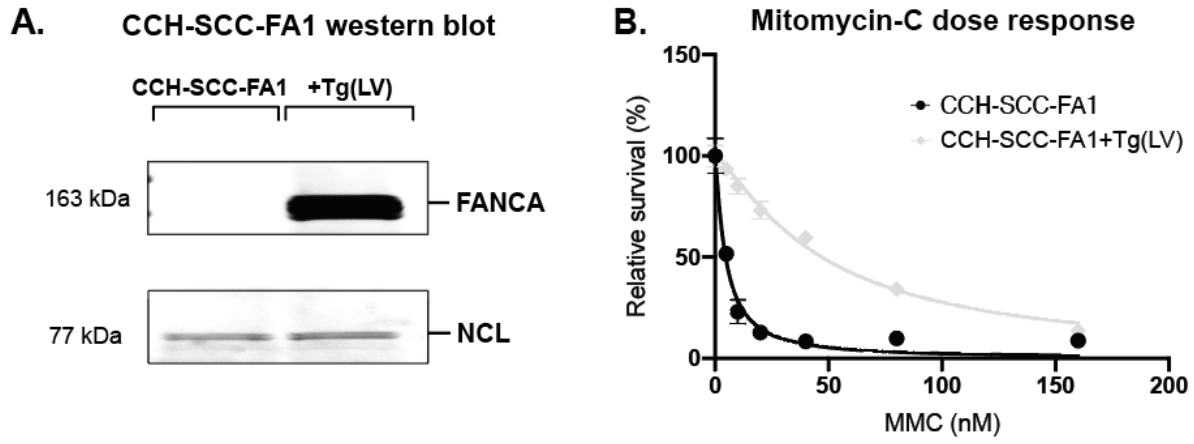

Figure 2: Verification of *FANCA* complemented clones. (A) Representative immunoblot result for detection of FANCA protein in 30 µg of whole cell lysates. (B) Assessment of cell survival by alamarBlue assay 4 days after continuous exposure to mitomycin C (MMC).

Protocols

1. 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: FANCA complemented CCH-SCC-FA1+Tg(LV) have a band indicating the presence of full-length wild type FANCA that is not present in the parent line.

2. 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: CCH-SCC-FA1+Tg(LV) demonstrates significantly greater resistance to MMC compared to the parent line.

References

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4. Roohollahi, K., de Jong, Y., Pai, G., Zaini, M. A., de Lint, K., Sie, D., Rooimans, M. A., Rockx, D., Hoskins, E. E., Ameziane, N., Wolthuis, R., Joenje, H., Wells, S. I., & Dorsman, J. (2022). BIRC2-BIRC3 amplification: a potentially druggable feature of a subset of head and neck cancers in patients with Fanconi anemia. *Scientific reports*, *12*(1), 45. https://doi.org/10.1038/s41598-021-04042-9
5. van Harten, A. M., Poell, J. B., Buijze, M., Brink, A., Wells, S. I., René Leemans, C., Wolthuis, R., & Brakenhoff, R. H. (2019). Characterization of a head and neck cancer-derived cell line panel confirms the distinct TP53-proficient copy number-silent subclass. *Oral oncology*, *98*, 53–61. https://doi.org/10.1016/j.oraloncology.2019.09.004

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