JHU-SCC-FaDuko (*FANCA*$^{-/-}$)
JHU-SCC-FaDuko+Tg (*FANCA*+ transgene-complemented from clonally derived *FANCA*$^{-/-}$ subline)

Description

Organism: *Homo sapiens*
Tissue: Pharynx
Disease: Head and neck squamous cell carcinoma
Age: 56Y
Gender: male
HPV status: negative
Synonyms: FaDu; FaDU; FADU

"JHU-SCC-FaDu" is a *FANCA*$^{+/+}$ sporadic head and neck squamous cell carcinoma (HNSCC) cell line distributed by the American Type Culture Collection (ATCC® HTB43™). The line was established from a punch biopsy of an hypopharyngeal tumor removed from a Hindu patient in 1968 (1). JHU-SCC-FaDu is not included in the resource but can be purchased through ATCC.

"JHU-SCC-FaDuko" is a clonally derived FANCA$^{-/-}$ cell line from JHU-SCC-FaDu. The FANCA knockout was generated by dual-gRNA cas9-mediated deletion targeting the N-terminus of the gene resulting in a loss of FANCA protein expression and function.

"JHU-SCC-FaDuko+Tg" is a clonally derived FANCA complemented subline of JHU-SCC-FaDuko. The FANCA complementing transgene was inserted into the chromosome 4 'safe harbor site' 231 (2).

Growth and selection media

JHU-SCC-FaDu was grown in Eagle's Minimum Essential media (Corning 10-010-CV), 10% fetal bovine serum (FBS, Hyclone Laboratories SH30071.03), and 1% penicillin-streptomycin (Gibco 15140122).

Antibiotic selection of JHU-SCC-FaDuko+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%	Multi-SNV/Indel
FAT1	23%	CNV Del/Frame Shift Del
CDKN2A	22%	Splice site
PIK3CA	21%	CNV Amp
NOTCH1	19%	
KMT2D	18%	
NSD1	10%	
CASP8	9%	
AJUBA	6%	
FBXW7	5%	
HRAS	4%	
PTEN	2%	

JHU-SCC-FaDu has somatic, homozygous nonsense mutations in *FANCM*, which has been confirmed by Sanger Sequencing (4).

Additional genomic characterization can be found in the resource manuscript (5), the Catalogue of Somatic Mutations in Cancer (COSMIC), and Broad Institute Cancer Cell Line Encyclopedia (CCLE).

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**
JHU-SCC-FaDu	**JHU-SCC-FaDu**	**JHU-SCC-FaDuko**	**JHU-SCC-FaDuko+Tg**
AMEL: none detected	AMEL: none detected	AMEL: none detected	AMEL: none detected
CSF1PO: 12	CSF1PO: 12	CSF1PO: 12	CSF1PO: 12
D13S317: 8,9	D13S317: 8,9	D13S317: 8,9	D13S317: 8,9
D16S539: 11	D16S539: 11	D16S539: 11	D16S539: 11
D5S818: 12	D5S818: 12	D5S818: 12	D5S818: 12
D7S820: 11,12	D7S820: 11,12	D7S820: 11,12	D7S820: 11,12
TH01: 8	TH01: 8	TH01: 8	TH01: 8
TPOX: 11	TPOX: 11	TPOX: 11	TPOX: 11
vWA: 15,17	vWA: 15,17	vWA: 15,17	vWA: 15,17

More extensive STR profiling data can be found the following publications (6,7).

General workflow

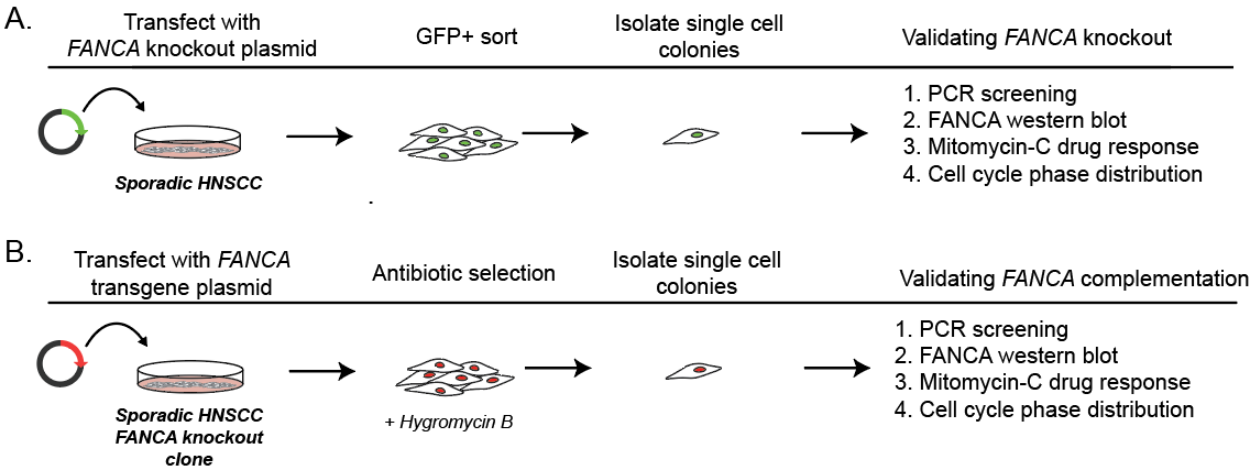

Figure 1: Workflow for generating, characterizing, and complementing *FANCA* isogenic cell line trios starting from sporadic HNSCC cell lines. (A) Generation of *FANCA* clonal knockouts. (B) Complementation of *FANCA* clonal knockouts.

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.

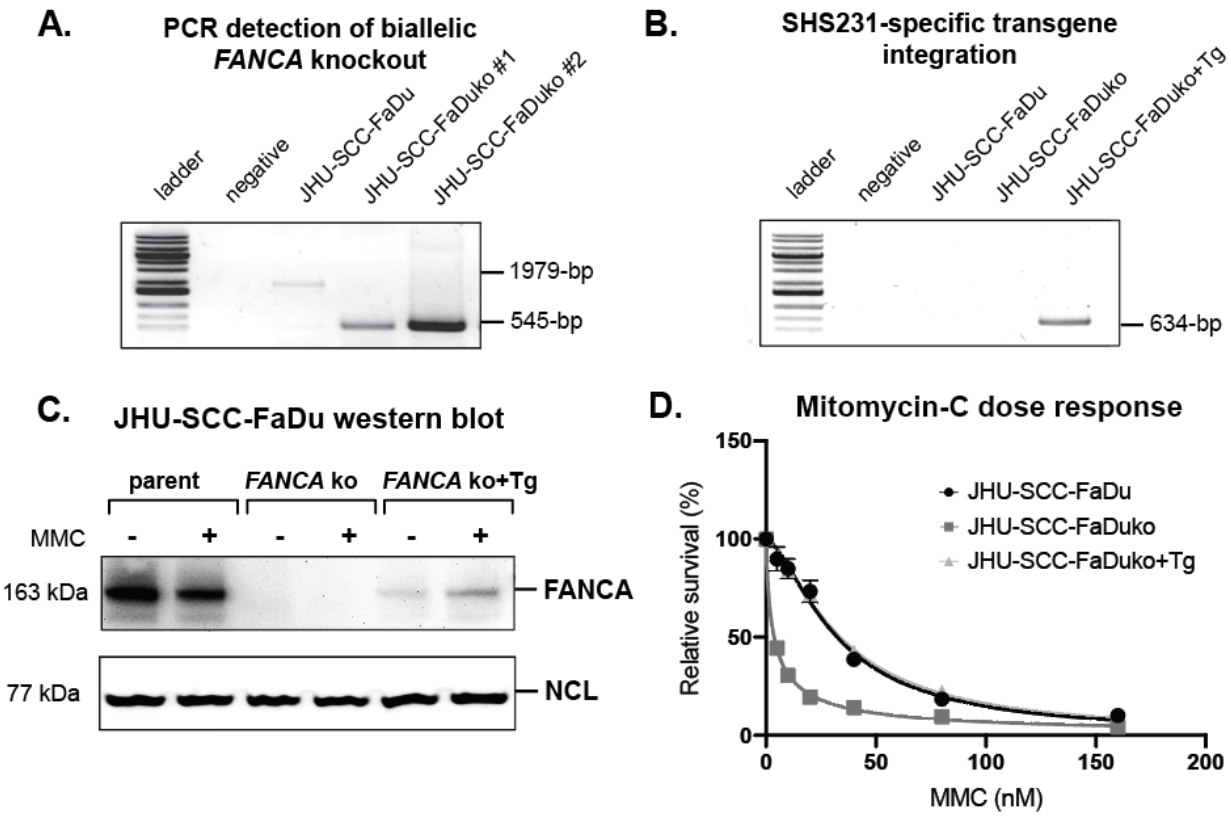

Figure 2: Verification of *FANCA* KO and *FANCA* complemented clones. (A, B) Representative PCR results for detection of (A) wild type FANCA and dual gRNA/cas9 mediated FANCA ko alleles and (B) SH231 site specific insertion of FANCA complementation vector, pSH231-EF1-FANCA-Hygro. (C) Representative immunoblot 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 JHU-SCC-FaDuko and JHU-SCC-FaDuko+Tg cells will show the roughly 545 base pair deletion band, while the JHU-SCC-FaDu 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, JHU-SCC-FaDuko+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: JHU-SCC-FaDu cells and FANCA complemented JHU-SCC-FaDuko+Tg have a band indicating the presence of full-length wild type FANCA, whereas in JHU-SCC-FaDuko 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: JHU-SCC-FaDuko demonstrate distinct hypersensitivity to MMC, while JHU-SCC-FaDuko+Tg will show restored resistance comparable to wild type JHU-SCC-FaDu cells.

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