The Fanconi Anemia Cancer Cell Line Resource

Datasheet for HNSCC cell line [trio]:

CAL33 (FANCA+/+)
CAL33-c11 (FANCA-/-)
CAL33-c11-FANCA (FANCA+/+)

Description

- CAL33 is a *FANCA+/+* sporadic head and neck squamous cell carcinoma (HNSCC) cell line distributed by German Collection of Microorganisms and Cell Cultures (DSMZ ACC 447). The line was established from squamous cell carcinomas of the tongue [1].

- *FANCA*-mutant cell line, CAL33-c11, was obtained from CAL33 cell line by CRISPR/Cas9 gene editing. Inactivating mutations were introduced in the *FANCA* gene by the generation of insertions/ deletions (indels) in parental cell line. This clone has frameshift indels in homozygosis and does not express FANCA protein.

- *FANCA*-mutant cell line, CAL33-c11, was complemented with wild type *FANCA* gene by retroviral infection to obtain CAL33-c11-FANCA.

The full description of the generation of the *FANCA* knockout CAL33 cells has been reported [2].

Source

Organism: Homo sapiens, human
Tissue: tongue
Disease: squamous cell carcinoma
Age: 69 years
Gender: male
HPV status: negative

Genomic data

The table list the top 25 driver genes in HNSCC and the mutational status in CAL33 cells [3 4].

Gene	Mutation	Gene	Mutation
TP53	+	*NFE2L2*	-
FAT1	+	*MYH9*	+
CDKN2A	+	*EPHA2*	-
NOTCH1	-	*TGFBR2*	-
PIK3CA	+	*KEAP1*	-
KMT2D	-	*HLA-A*	-
NSD1	-	*HLA-B*	+
CASP8	+	*ARID2*	-
HUWE1	-	*KDM6A*	-
FBXW7	-	*ZNF750*	-
EP300	+	*CUL3*	-
AJUBA	-	*FLNA*	-
HRAS	-		

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Additional information on cell line mutations and datasets on loss of function and drug profiling screen data can be found at the following links:

CCLE project genomic mutations data:
https://portals.broadinstitute.org/ccle/page?cell_line=CAL33_UPPER_AERODIGESTIVE_TRACT

COSMIC:
https://cancer.sanger.ac.uk/cell_lines/sample/overview?id=753541

Depmap Portal:
https://depmap.org/portal/cell_line/ACH-000518?tab=mutation

Genomics of Drug Sensitivity in Cancer
https://www.cancerrxgene.org/cellline/CAL-33/753541

Quality Control Testing

- Cells were tested for *Mycoplasma*. All cell lines came back negative for all.
- Cells were sent to CIMA Lab Diagnostics, at the Universidad de Navarra (Spain) to be authenticated using the AmpFLSTR® Identifiler® Plus PCR Amplification Kit.
- A Human species-specific STR marker profile was established for the cell line duos and used for comparative analysis with available published profiles to confirm their unique identity. The unique marker results from prior test and our recent analysis are listed in STR profiling panels below.

STR profiling

STR: DMSZ CAL33	STR: CIMA CAL33	STR: CIMA CAL33-c11
	D8S1179: 13	D8S1179: 13
	D21S11: 29,30	D21S11: 29,30
D7S820: 8,10	D7S820: 8,10	D7S820: 8,10
CSF1PO: 11,12	CSF1PO: 11,12	CSF1PO: 11,12
	D3S1358: 17	D3S1358: 17
TH01: 9,9.3	TH01: 9,9.3	TH01: 9,9.3
D13S317: 8,13	D13S317: 8,13	D13S317: 8,13
D16S539: 11,11	D16S539: 11	D16S539: 11
	D2S1338: 20,25	D2S1338: 20,25
	D19S433: 14,15.2	D19S433: 14,15.2
vWA: 17,17	vWA: 17	vWA: 17
TPOX: 8,8	TPOX: 8	TPOX: 8
	D18S51: 14	D18S51: 14
AMEL: X,Y	AMEL: X,Y	AMEL: X,Y
D5S818: 11,12	D5S818: 11,12	D5S818: 11,12
	FGA: 21,22	FGA: 21,22

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For the more detailed STR profiling data, please check the following publications:

1. Zhao M, et al. Assembly and initial characterization of a panel of 85 genomically validated cell lines from diverse head and neck tumor sites. Clin Cancer Res. 17(23): 7248-64, 2011. DOI: 10.1158/1078-0432.CCR-11-0690

2. Yu M, et al. A resource for cell line authentication, annotation and quality control. Nature. 520(7547): 307-11, 2015. DOI: 10.1038/nature14397

Protocols

Refer to the attached DMSZ data sheet for additional information on complete culture media, how to handle, expand and cryopreserve these cells.

A more detailed description of the protocols to generate isogenic pairs and phenotype characterization has been published [2].

The following molecular, biochemical and phenotypic assays were used to verify the generated *FANCA* knockout.

Graphical workflow

Figure 1.

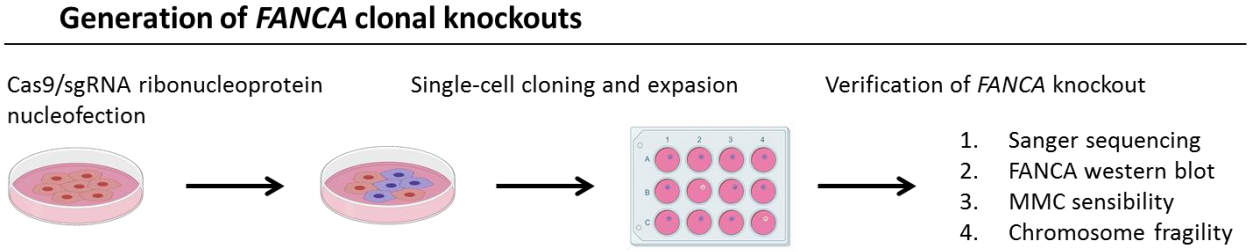

Figure 1: Workflow for generating and characterizing *FANCA*-mutant cells starting from sporadic HNSCC cell line CAL33.

Figure 2.

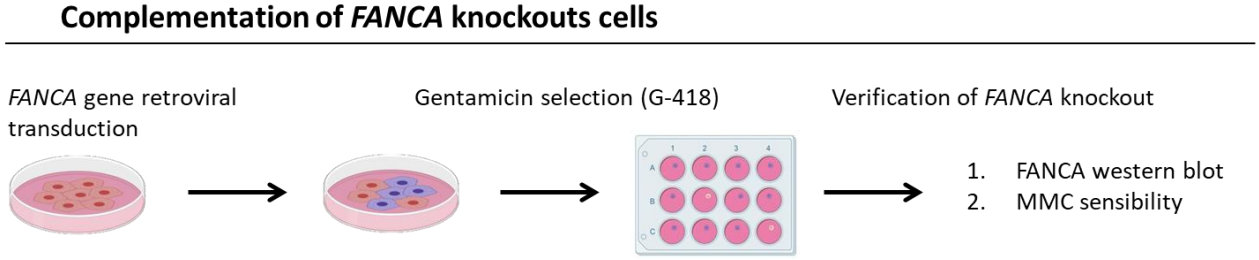

Figure 2: Workflow for complementation of FANCA-mutant cell line, CAL33-c11.

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Molecular analysis using PCR/Sanger sequencing

For detection of FANCA wild type (WT) and knockout (KO) alleles:

Primers used:

- Forward primer sequence: 5' TGCTCCTTTTGTGTCATGGGA 3´
- Reverse primer sequence: 5' TGCTGGTGTCTTACTCTCTGC 3´
- Sanger seq primer sequence: 5'-CCTTTGCATCTATTCTCCCCGT-3'

PCR conditions:

- 95ºC, 10 minutes
- Number of cycles: 40 — Denaturation: 95ºC, 20 seconds; Annealing: 60ºC, 20 seconds; Extension: 72ºC, 30 seconds
- 72ºC, 30 seconds

Expected results:

The PCR products using these primer sequences include a 422 base pair (bp) intact band (FANCA WT) and 405 bp edited band (CAL33-c11). Sanger sequencing performed on the PCR with the indicated primer will result in the following chromatograms:

CAL33-c11

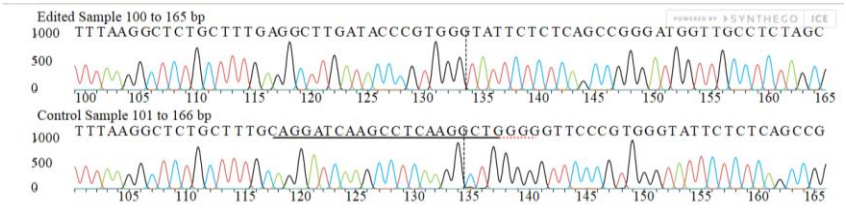

Figure 3. Chromatograms of parental CAL33 cells (Control samples) and *FANCA*-ko CAL33-c11 (Edited Sample) after PCR amplification and Sanger sequencing on genomic DNA using Forward, Reverse and Sanger seq primers indicated above.

Biochemical analysis using a FANCA Western Blot

Antibodies used:

FANCA primary antibody: Rabbit polyclonal, Abcam, ab5063

Secondary antibody: Donkey anti-rabbit IgG, HRP conjugated, Amersham, NA934

Expected results:

The CAL33 and CAL33-c11-FANCA cells will have a band indicating the presence of full-length wild type *FANCA*, whereas in CAL33-c11 cells will be no detection of the full length wild type FANCA protein due to the deletion in the *FANCA* gene.

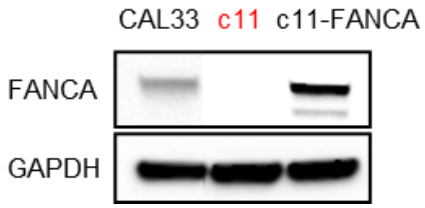

Figure 4. Representative immunoblot result for detection of FANCA protein in 40 µg of whole cell lysates from CAL33), CAL33-c11 (c11) and CAL33-c11-FANCA (c11-FANCA) cells.

Phenotypic analysis using a Mitomycin C sensitivity assay

Reagents:

- Mitomycin C (MMC) from Streptomyces caespitosus (Sigma Cat. M0503)

Procedure:

1. 100,000 cells are seeded per well in 6-well plates.
2. Cells are treated for 1 hour with increasing concentrations of MMC ranging from 0nM to 38.4µM in PBS.
3. Incubate for 5 days.
4. Cells are stained with crystal violet and then eluted with 33% acetic acid to quantify cell viability.
5. Cell viability is determined using the Genius Pro (Tecan) microplate reader.

Expected results:

CAL33-c11 will show hypersensitivity to MMC when compared to wild type CAL33 and complemented CAL33-c11-FANCA cells.

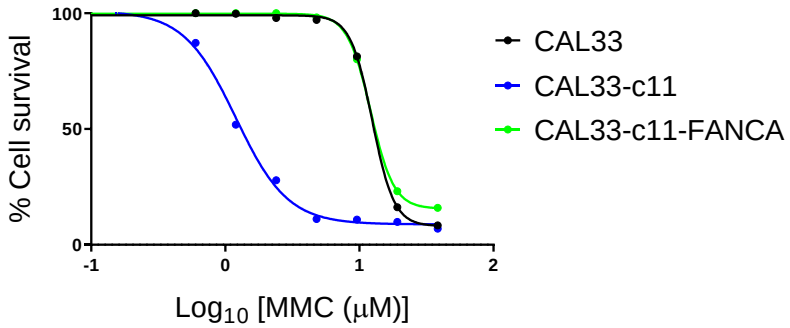

Figure 5. Assessment of cell survival 5 days after 1 hour of exposure to mitomycin C (MMC).

References

1. Gioanni, J. *et al.* Two new human tumor cell lines derived from squamous cell carcinomas of the tongue: establishment, characterization and response to cytotoxic treatment. *Eur J Cancer Clin Oncol* **24**, 1445-1455 (1988).
2. Errazquin, R. *et al.* Generating New FANCA-Deficient HNSCC Cell Lines by Genomic Editing Recapitulates the Cellular Phenotypes of Fanconi Anemia. *Genes (Basel)* **12** (2021).
3. Ghandi, M. *et al.* Next-generation characterization of the Cancer Cell Line Encyclopedia. *Nature* (2019).
4. Bailey, M.H. *et al.* Comprehensive Characterization of Cancer Driver Genes and Mutations. *Cell* **173**, 371-385 e318 (2018).

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