Characterization of an oncogenic isoform of *TP53*: Δ160p53

Ana Catarina Ramalho[1]; Filipa Rita[1]; Jingyuan Zhao[2]; Maria López-Iniesta[2]; Rafaela Lacerda[1]; Luísa Romão[3]; Marco M. Candeias[4]

[1]MaRCU — Molecular and RNA Cancer Unit; Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal; BioISI — BioSystems & Integrative Sciences, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal;
[2]MaRCU — Molecular and RNA Cancer Unit; Graduate School of Medicine, Kyoto University, Kyoto, Japan;
[3]Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal; BioISI — BioSystems & Integrative Sciences, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal;
[4]MaRCU — Molecular and RNA Cancer Unit; Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal; BioISI, FCUL, Universidade de Lisboa; Graduate School of Medicine, Kyoto University, Kyoto, Japan

Ciências ULisboa

Instituto Nacional de Saúde Doutor Ricardo Jorge

BioISI Biosystems and Integrative Sciences Institute

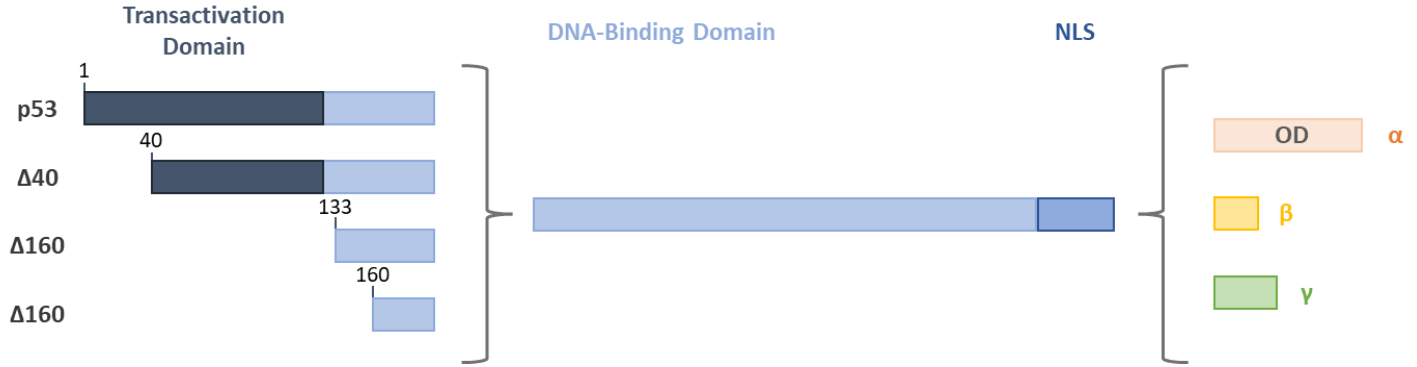
MaRCU The Molecular and RNA Cancer Unit

Kyoto University Founded 1897

Introduction

The *TP53* gene

- **Tumor suppressor gene**.
- Encodes for **12 protein isoforms**.



• p53α is a transcription factor kept at low levels in normal conditions.

• In response to stress, such as DNA damage, p53α is activated to promote cell cycle arrest, apoptosis or senescence.

Guardian of the genome

• The *TP53* gene is mutated in over half of all human cancers (mostly missense mutations), leading to:

- Loss of wild-type tumor suppressor functions;

- Gain of new functions that actively drive tumorigenesis, invasion and metastasis.

Proto-oncogene

Δ160p53α

• This isoform is overexpressed in cancer cells and presents oncogenic functions.

• It is proposed that Δ160p53α is a proto-oncogene and the missense mutations commonly found in tumor cells promote oncogenesis by favoring the translation of this isoform.

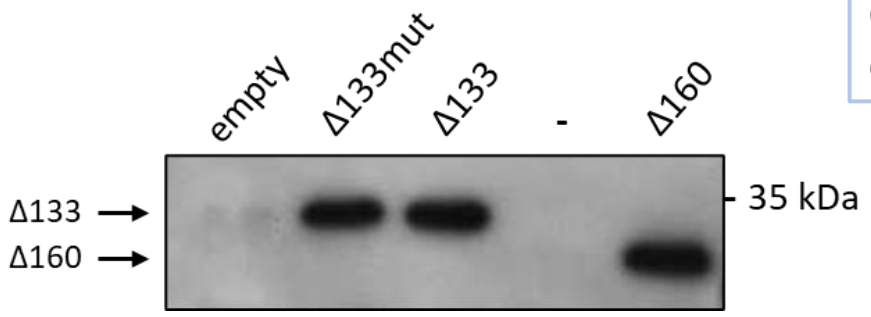
Anchorage-independent growth of cells expressing the p53 isoforms Δ133 and Δ160

Soft agar colony formation assay

Stable clones expressing the p53 isoforms:

Saos-2
p53-null osteosarcoma cell line
↓
Transfection
p53 short isoforms
↓
Selection
Geneticin 500 µg/ml
↓
Expansion

Construct name	Description
Δ133	Δ133p53 with part of its 5'UTR sequence that corresponds to the beginning of exon 5
Δ133mut	Δ133p53 with kozak sequence; ATG160 and ATG169 are mutated
Δ160	Δ160 with kozak sequence
Empty	empty pcDNA3.1+ vector



Western blot of Saos-2 cells stably transfected with p53 isoforms.

Confirmation that the cells stably express the transfected isoforms.

Anchorage-independent growth of cells expressing the p53 isoforms Δ133 and Δ160

Soft agar colony formation assay

Plating of bottom layer

↓

Plating of upper layer
Saos-2 stable cells

↓

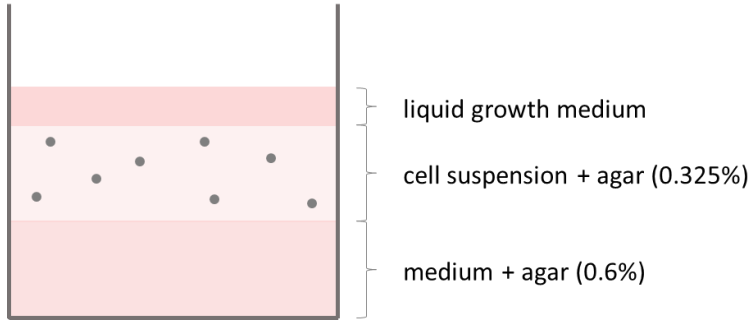
Top with growth medium

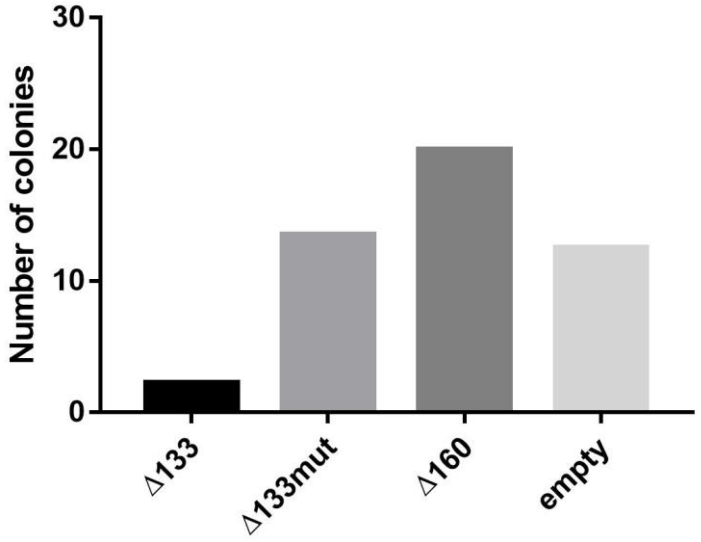
↓ 21 – 28 days

Staining
0.1% crystal violet

↓

Image Acquisition
and
Colony counting





Soft agar colony formation assay of Saos-2 stable cells expressing p53 isoforms. Results from one experiment with 3000 cells (2 replicates).

- The higher number of colonies seen in Δ160p53 cells indicates that it favors anchorage-independent growth.
- More assays are needed to confirm the results for the different isoforms.

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