The centrosomal localization of p53 is critical for spindle pole integrity in human nontransformed cells but not in cancer cells

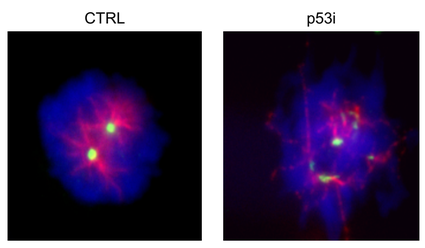
76

I. Virdia, C. Contadini, G. Di Rocco, L. Monteonofrio, S. Soddu
Department of Research, Advanced Diagnostic, and Technological Innovation - Translational Research Area, Regina Elena National Cancer Institute, Rome, Italy

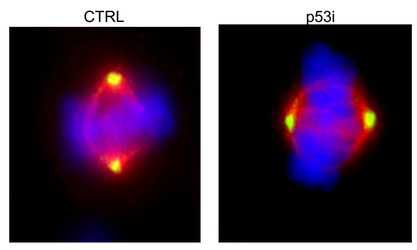
ABSTRACT

During mitosis, two newly formed centrosomes assemble a bipolar spindle and ensure faithful segregation of chromosomes. We recently showed that, in untransformed human cells, integrity of centrosomes in M phase and proper spindle formation require p53 mitotic centrosomal localization. On the contrary, human tumor cells can survive in the absence of p53. To investigate how cancer cells evade centrosome-associated p53 control, we will generate a normal human cell line in which it will be possible to remove p53 in a fast, efficient, reversible and inducible way using the auxin-inducible degron system (AID) and the CRISPR-Cas9. We verified that degron tag both at N- and C-terminal does not affect p53 transcriptional activity and centrosomal localization. We also verified the AID system activity and observed that the treatment with the auxin, in the presence of its receptor, is able to degrade the p53 protein only when it is fused with the degron tag. Based on these results, we will tag endogenous p53 with degron sequence in nontrasformed human cells. This will provide a cell model to study how human tumor cells evade loss of spindle pole integrity and prevent mitotic catastrophe.

In non-transformed human cells p53-MCL is required to maintain centrosome and spindle pole integrity



Cancer human cells evade the *centrosome-associated p53 control*



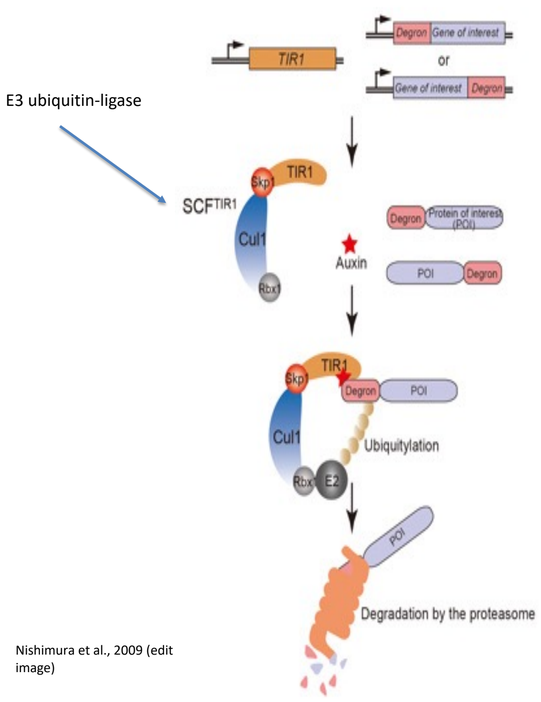
Centrosome-loss, leaving p53 orphan of its mitotic centrosomal localization, promotes the formation of discrete foci of Ser15-phosphorylated p53 that, by recruiting 53BP1 triggers its own further activation and subsequent cell cycle arrest

These data support the hypothesis that p53-MCL contributes to the "mitotic surveillance pathway"

- ✓ **How does p53-Mitotic Centrosomal Localization (p53-MCL) control the spindle pole integrity in human cells?**
- ✓ **How do human tumor cells escape p53-MCL check-point?**

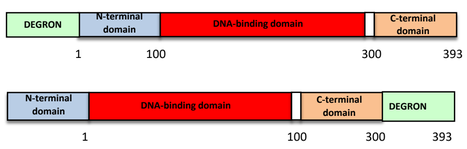
Step by step…

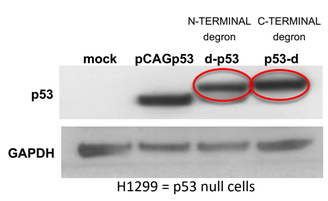
Auxin Inducible Degron system (AID) to degradate degron-tagged p53



Nishimura et al., 2009 (edit image)

1) CLONING OF A NEW TAG FOR p53: THE DEGRON

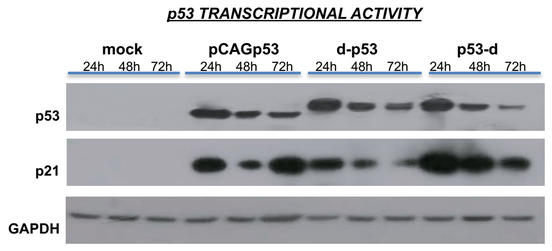




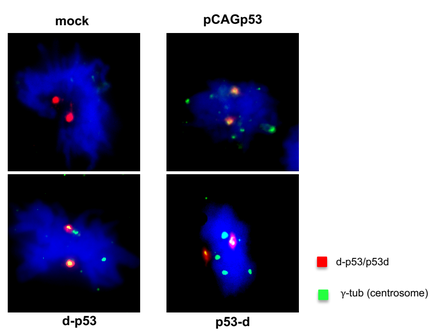
H1299 = p53 null cells

2) p53 FUSION PROTEIN ACTIVITY

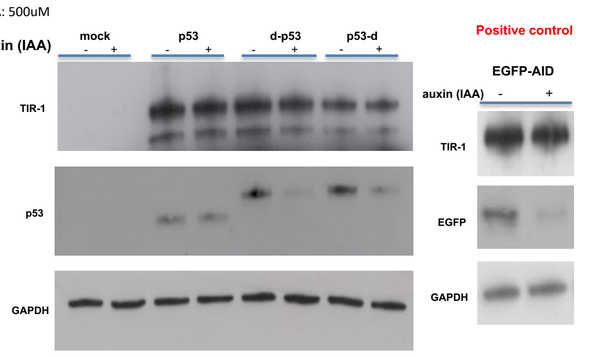
p53 TRANSCRIPTIONAL ACTIVITY



p53 CENTROSOMAL LOCALIZATION

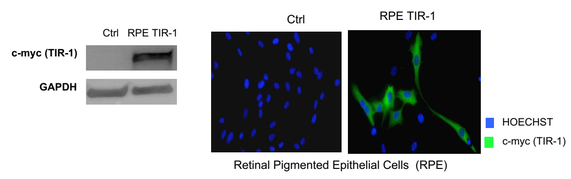


3) AID ACTIVITY



H1299 = p53 null cells

RPE-Tir1 Stable cell line

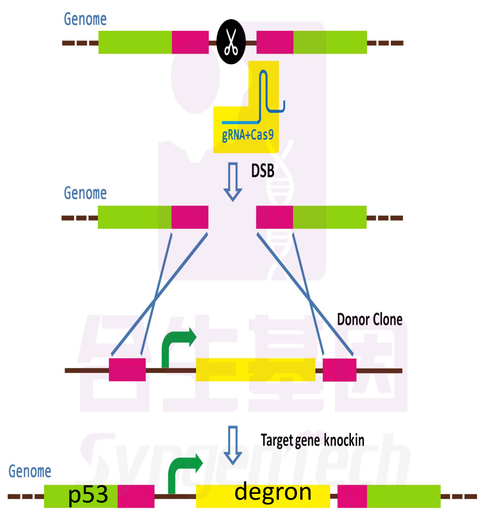


Retinal Pigmented Epithelial Cells (RPE)

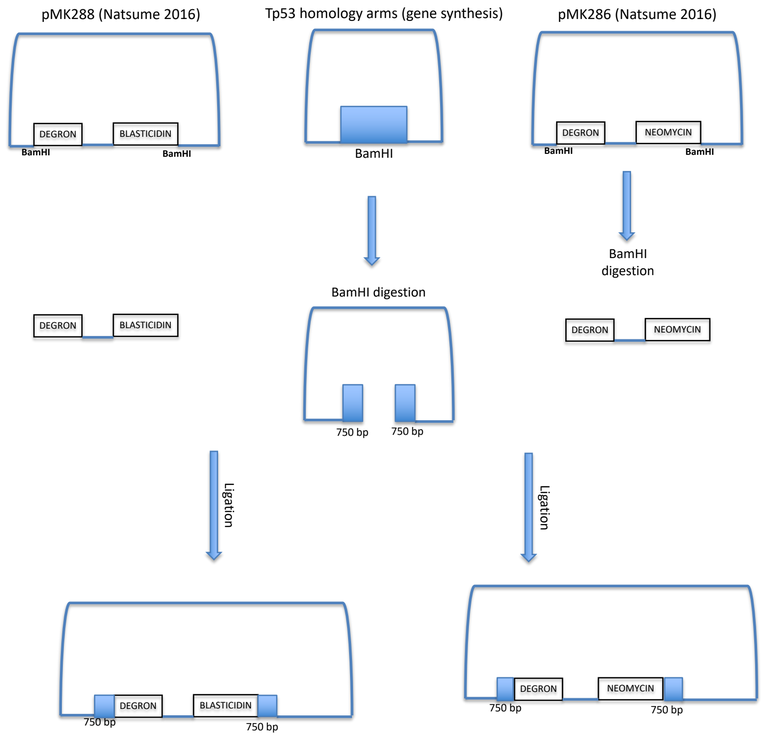
Work in progress

Experimental design

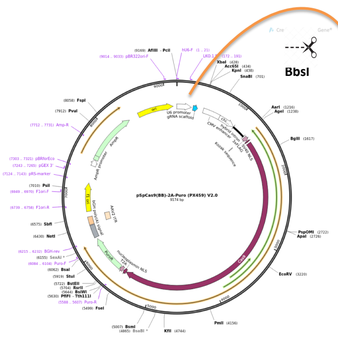
CRISPR-Cas-9 genome editing to tag endogenous



1) Donor plasmids



2) CAS-9/gRNA



gRNA sequence: ACGCACACCUAUUGCAAGCA

Forward oligo: CACCGACGCACACCAGCAAGCA
Reverse oligo: AAACTGCTTGCTGGTGTGCGTC

pSpCas9(BB)-2A-Puro (PX459) v2.0 (Natsume 2016)

3) Knock in