Accidental mini p53 isoform discovery could be key to elucidating new functions of p53 gene

Shrutee N. Parkar, Masatoshi Hagiwara, Marco m. Candeias

MaRCU, Grad. Sch. of Med., Kyoto Univ
Dept. of Anat and Dev. Biol., grad. Sch. of Med., Kyoto Univ.
Dept. of Hum. Gen., NIH Dr. Ricardo Jorge

Introduction:

p53 gene plays an important role in cells by regulating cell stemness, division, differentiation, senescence and death; response to stress, infection and disease; reproduction, metabolism, regeneration, aging and life span, not only by the full-length (FL) p53 protein but also with the help of the p53 mRNA and the alternatively translated shorter p53 isoforms.

Background:

Evidence has accumulated that p53, a prototypical tumor suppressor, may also influence other aspects of organismal function and aging. Here we identified a new isoform, *mini p53 isoform* (MPI), the smallest p53 isoform identified so far. We have confirmed the sequence and expression mechanisms for this new isoform and we have investigated its expression in human cancers. We will discuss our insights and early evidences of MPI's role in cellular and organismal function.

Figure 1.

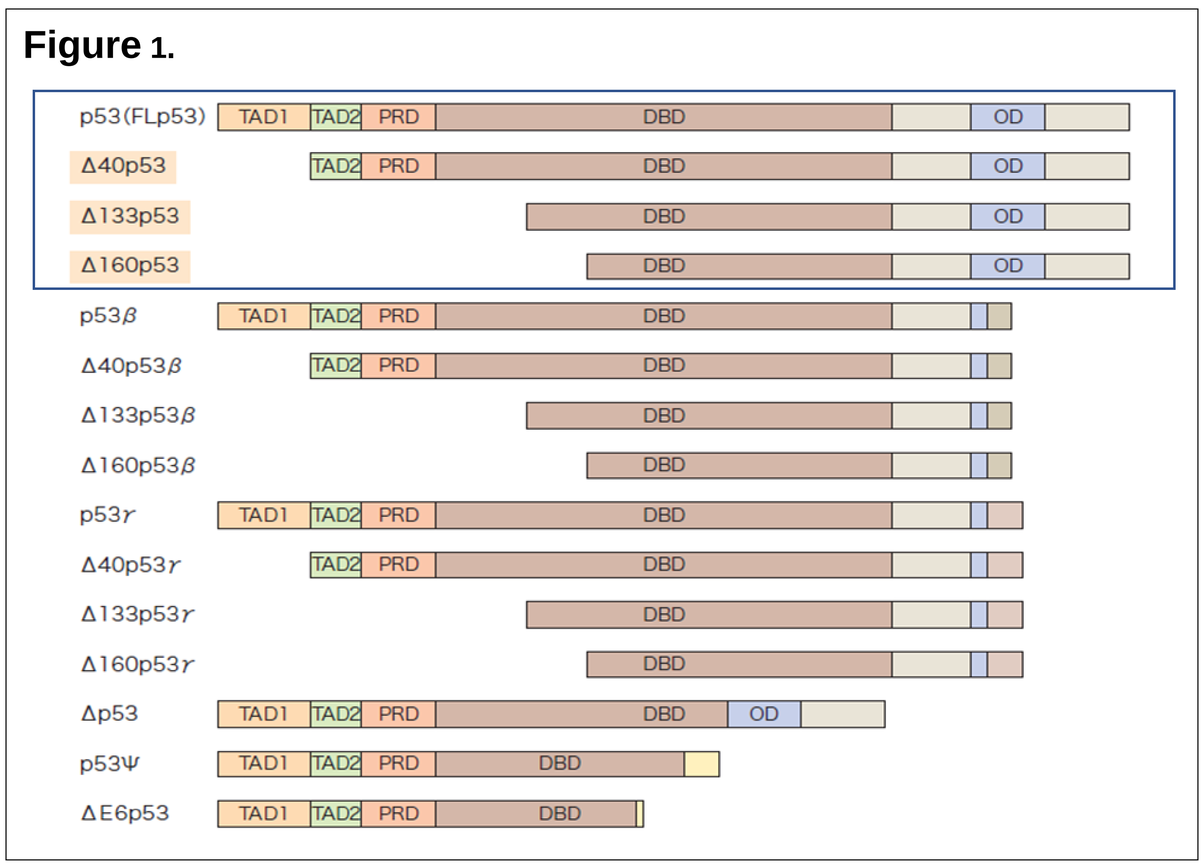


Figure 1. List of Human p53 protein isoforms described so far.

Purpose:

- To investigate MPI, to accumulate evidence of it's involvement in new functions of p53 protein.
- Create a MPI stable cell line
- Induce senescence in the MPI stable cell line and check for SA-β-Gal activity .

Results:

Figure 2:

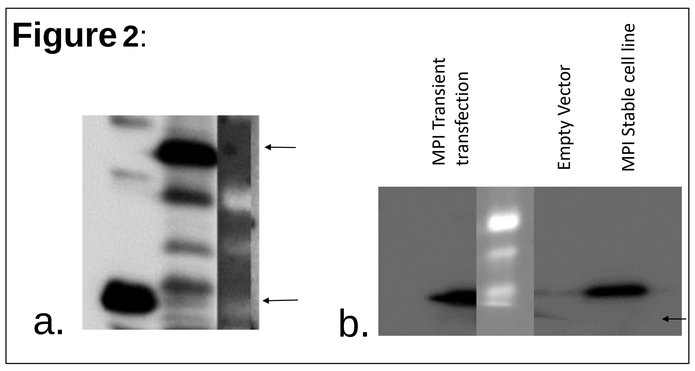
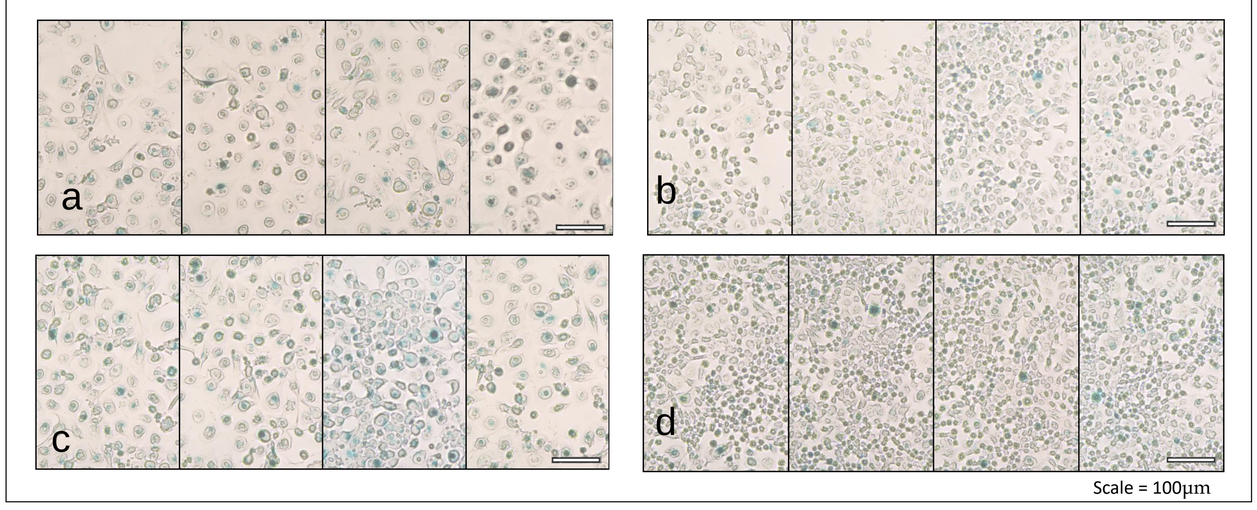


Figure 2: a. Size of new isoform confirmed with WB. b. MPI stable cell line in H1299 cells passage 13. Antibodies: 1:8000 CM-1 (polyclonal P53)

Figure 3. Beta Galactosidase assay



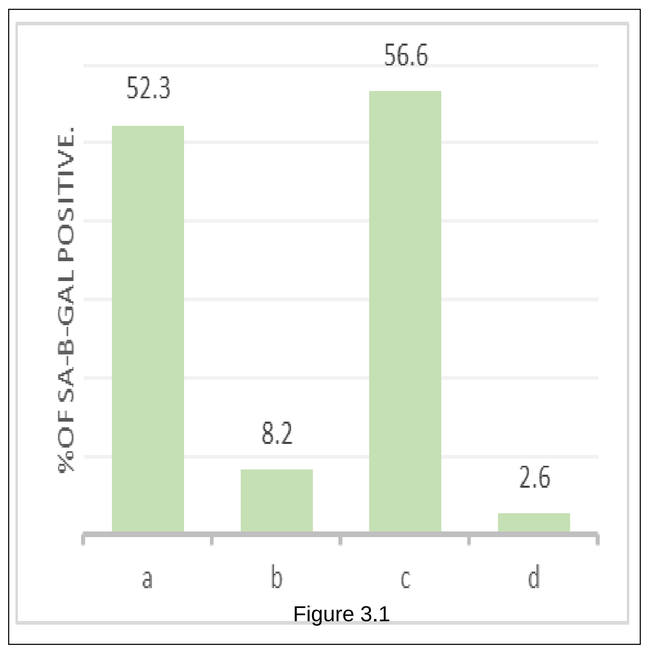
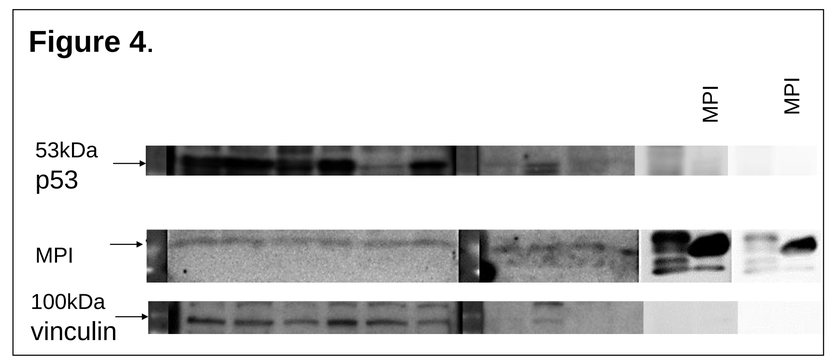


Figure 3.1

Figure 3. Senescence was induced with Etoposide(ETO)(5mM) 1/1000 dilution (48hr drug treatment) Stable cell line of MPI and V1 (empty vector) in h1299 cells, passage 16.
(data is average of 4 images manually counted and calculated)
SA-β-Gal staining (16hrs) following standard protocol.
a. MPI ETO treatment b. MPI DMSO c.V1 ETO d. V1 DMSO
Figure 3.1 Graph showing % of SA-β-Gal activity.

Figure 4. Western Blot done following standard protocol, of proteins extracted from Human Breast cancer biopsies (Czech Republic)

Figure 4.



Conclusion:

- The new isoform could affect senescence in cancer cells.
- It can be detected in the human cancer tissues and further investigation is on the way.

For more work from our lab please visit poster number **1LBA-064 : Shorter p53 isoforms: the new-found link between cancer and p53 protein stabilization**

LOPEZ INIESTA Maria Jose

Visit MaRCU website, areap53.com

aRCU
The Molecular and RNA Cancer Unit

Send an e-mail to:

shrutee1991@gmail.com