高度保存癌原遺伝子である p53 イソフォム：Δ160

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Part of this project has now been published/preprinted as follows:

1. López-Iniesta MJ, Parkar SN, Ramalho AC, Lacerda R, Costa IF, Zhao J, Romão L, Candeias MM. Conserved Double Translation Initiation Site for Δ160p53 Protein Hints at Isoform's Key Role in Mammalian Physiology. Int J Mol Sci. 2022 Dec 13;23(24):15844. doi: 10.3390/ijms232415844. PMID: 36555484; PMCID: PMC9779343.

2. Maria José López-Iniesta, Rafaela Lacerda, Ana Catarina Ramalho, Shrutee N Parkar, Ana Marques-Ramos, Bruna Pereira, Lina Miyawaki, Jun Fujita, Roman Hrstka, Luísa Romão, Marco M Candeias. Internal Translation of p53 Oncoproteins During Integrated Stress Response Confers Survival Advantage on Cancer Cells. bioRxiv 2023.03.03.531004; doi: https://doi.org/10.1101/2023.03.03.531004

Another part of the work has been submitted and we are waiting publication.

Some of the main findings are described below:

1. Define and characterize the RNA structures (IRESs) responsible for the specific translation of Δ133p53 and Δ160p53 isoforms:

We thoroughly examined the newly identified IRES in the short p53 mRNA (P2 mRNA). Hundreds of control experiments were executed in order to validate its capacity to induce translation initiation of D160p53 protein isoform in a 5'-cap independent manner, including bicistronic reporter constructs, promoterless constructs (which showed no cryptic promoter activity), RT-PCR using different primers (which failed to detect alternative splicing) and different siRNA (also showing no alternative RNA products). Using luciferase reporter constructs with could establish the limits of the IRES.

We also established, through chemical probing and primer extension assays, the first RNA structures of p53's short P2 mRNA (an mRNA that initiates from a TSS in intron 4). These structures include the structure of IRESD160.

2. Test the impact of stress conditions on IRES function and on the expression of p53 isoforms:

Integrated stress response (ISR) is a survival pathway frequently activated in cancers, marked by the phosphorylation of eukaryotic initiation factor 2alpha (eIF2α) and a defined reprogramming in mRNA translation. We identified ISR as a powerful trigger of p53 oncogene, leading to the induction of not only Δ160p53 but also Δ133p53, another protein variant of the p53 gene. Upon ISR the two isoforms were translated internally from p53 full-length (FL) transcript through an internal regulator of expression site (IRES) located in the vicinity of codon 160.

3. Investigate how much of the effect of p53 on tumor onset and progression is due to increased Δ160p53 expression and IRES activation:

We used cells stably expressing D160p53 in soft-agar colony formation assays. D160p53 expression alone was sufficient to induce tumor growth, confirming the oncogenic nature of D160IRES and D160p53 protein isoform.

4. Target the oncogenic functions of p53 in cells and mouse models by inhibiting Δ160IRES function and Δ160p53 expression using antisense oligos:

Lastly, in vivo morpholino oligos (Gene-tools) designed against D160IRES specifically reduced D160p53 and D133p53 expression with little or no effect on the expression of full-length p53 protein and successfully reduced cancer cell survival and growth.

Concluding, the project was very successful, considering that we accomplished what we set to do and were rewarded with extra findings as well.

Next, we want to investigate everything about the D160p53 protein isoform, one of the oldest p53 isoforms and most commonly mutated oncoprotein in cancer, in order to identify a druggable target for therapy. We possess the leading edge and hope to obtain the funding.