PROJECT

Supervisor: Davide Mazza

Title: Single molecule analysis and targeting of oncosupressive dynamical switches.

Curriculum: CMB

Link to the personal page of the University or relevant hospital site website: https://www.unisr.it/en/docenti/m/davide-mazza

Description of the Project (max 3,000 characters including spaces)

Background/gap of knowledge

The tumor suppressor p53 is a transcription factor that – in response to the detection of DNA damage- controls the expression of genes involved in DNA repair, cell cycle arrest and apoptosis[1]. Thus, depending on the context, the activation of p53 can skew the cell fate towards opposite outcomes, survival or death[2,3]. Understanding the mechanisms underlying this choice would allow designing strategies to modulate cancer cell chemosensitivity.

Rationale and hypothesis

We used advanced live cell-microscopy to measure – with single molecule sensitivity - the transcription dynamics of *CDKN1A* – a canonical target gene of p53 encoding for the p21 protein, involved in cell cycle arrest. The experiments provided some surprises: upon p53 activation, CDKN1A transcription is activated as a switch, with individual cells transitioning suddenly from an "off" to an "on" state, and then remaining active for hours. We hypothesize that targeting the mechanisms controlling these transcriptional switches will be promising for future therapies aimed at blunting or hyperactivating p53 responses.

Objectives and specific aims

The proposed project aims ad identifying the biophysical and molecular mechanisms underlying such 'switch-like' activation. To reach this overarching goal, the student will first identify if digital activation of transcription is common among p53 targets, or if it only occurs for specific subclasses of them (e.g. genes involved in DNA repair, vs. apoptosis). Next, degron-induced perturbation of p53 levels will be used to define the biophysical model underlying

switch-like activity, and its relationship with p53 dynamics. Finally, the molecular mechanisms controlling these switch will be investigated by a combination of bottom-up, hypothesis driven experiments and top-down, genomics based investigation. High content screening of epigenetic drugs that could be used to perturb p53-mediated transcriptional activity, will also be considered, depending on the student interest.

To reach these goals, the student will combine CRISPR/Cas9 gene editing, advanced microscopy (live-cell imaging of transcription[4,5], single molecule tracking[6–8]) and cutting-edge genomics (single molecule footprinting[9–11], spatial transcriptomics).

Expected outcomes

The findings of the student will be fundamental to characterize p53-mediated transcriptional activation and identify novel strategies to control cellular response to genotoxic chemotherapy in p53-wt cancers.

Skills that the student should acquire (max. 600 characters including spaces):

The PhD student involved in our project will have the opportunity to be trained in a stimulating multidisciplinary environment on cutting-edge approaches in cellular and molecular biology, quantitative biology and advanced microscopy, including MS2-based measurement of transcriptional dynamics. The student will also use gene editing approaches such as CRISPR/Cas9 to generate other reporters of p53-mediated transcription. Training on advanced genomics (Single molecule footprinting) will be carried out in collaboration with Arnaud Krebs (EMBL, Heidelberg).

References (max. 15)

1.   Lane, D. & Levine, A. p53 Research: The Past Thirty Years and the Next Thirty Years. Cold Spring Harbor Perspectives in Biology 2, a000893–a000893 (2010).

2.   Purvis, J. E., Mock, K. W. K. C., Batchelor, E., Loewer, A. & Lahav, G. p53 Dynamics Control Cell Fate. Science 336, 1440–1444 (2012).

3.   Hafner, A., Bulyk, M. L., Jambhekar, A. & Lahav, G. The multiple mechanisms that regulate p53 activity and cell fate. Nat Rev Mol Cell Biol 20, 199–210 (2019).

4.   Zambrano, S. et al. First Responders Shape a Prompt and Sharp NF-κB-Mediated Transcriptional Response to TNF-α. iScience 23, 101529 (2020).

5.   Hafner, A. et al. Quantifying the Central Dogma in the p53 Pathway in Live Single Cells. Cell Systems 10, 495-505.e4 (2020).

6.   Mazza, D., Abernathy, A., Golob, N., Morisaki, T. & McNally, J. G. A benchmark for chromatin binding measurements in live cells. Nucleic Acids Research (2012) doi:10.1093/nar/gks701.

7.   Loffreda, A. et al. Live-cell p53 single-molecule binding is modulated by C-terminal acetylation and correlates with transcriptional activity. Nat Commun 8, 313 (2017).

8.   Mazzocca, M. et al. Chromatin organization drives the search mechanism of nuclear factors. Nat Commun 14, 6433 (2023).

9.   Kreibich, E., Kleinendorst, R., Barzaghi, G., Kaspar, S. & Krebs, A. R. Single-molecule footprinting identifies context-dependent regulation of enhancers by DNA methylation. Molecular Cell 83, 787-802.e9 (2023).

10.   Krebs, A. R. et al. Genome-wide Single-Molecule Footprinting Reveals High RNA Polymerase II Turnover at Paused Promoters. Molecular Cell 67, 411-422.e4 (2017).

11.   Sönmezer, C. et al. Molecular Co-occupancy Identifies Transcription Factor Binding Cooperativity In Vivo. Molecular Cell 81, 255-267.e6 (2021).