Super-resolution microscopy to visualize microtubules and nuclear lamin organization at mitotic exit.

In a recent study conducted in the laboratory of Dr Giulia Guarguaglini at the Institute of Molecular Biology and Pathology of CNR (IBPM-CNR, Rome), it has been shown that the microtubule-associated protein TPX2, well-characterized as a key regulator of mitotic spindle assembly and function, plays an unexpected role in cytoskeleton microtubule (MT) remodeling and nuclear reassembly at the mitosis-to-interphase transition (*Naso et al., 2020*).
The effects of TPX2 overexpression were examined in hTERT RPE-1 cells, a non-transformed cellular background. The authors observed that excess TPX2 causes defects in mitotic spindle assembly and delay in mitotic progression at the level of prometa/metaphase. Furthermore, comparing the effect of full length TPX2 with a truncated form unable to bind Aurora-A, a mitotic kinase properly activated, localized and stabilized by TPX2 interaction, they noticed that these defects are more severe when the full length TPX2 is overexpressed, indicating that the ability to interact with Aurora-A plays a crucial role.
In addition, they observed that TPX2 overexpression induces a peculiar Aurora-A-interaction-independent phenotype at telophase, when endogenous TPX2 is physiologically degraded by the proteasome. Abnormal intracellular bridges in late telophase are present: aberrantly stable MTs interfere with cytoskeleton disassembly and proper nuclear reconstitution at mitotic exit, leading to doughnut-shaped nuclei in daughter cells and preventing the assembly of a continuous lamin B1 network. Super-resolved images enable to visualize a strongly compromised lamin B1 structure, with large fenestrations, interconnected to MTs, in TPX2 overexpressing telophases (Figure A) and in the following interphase cells (Figures B, C).
Overall, these observations link TPX2 overexpression to the loss of nuclear integrity and highlight the importance of controlling TPX2 levels at ana-telophase for regulating MTs and nuclear reformation, as well as for chromosomes and organelles reorganization, in order to promote a correct transition from mitosis to the subsequent G1 phase (for further details see *Naso et al., 2020*).

It is noteworthy that high levels of TPX2 are reported in tumors, often within signatures of mitotic genes such as Aurora-A. This study points out that TPX2 overexpression per se does not cause appreciable chromosome segregation errors that could give rise to genetically unbalanced daughter cells, a hallmark of cancer. This observation leads to speculate that TPX2 may have a role in the maintenance, rather than in the onset, of chromosomal instability in cancer cells, by fueling mitotic errors through the impairment of correct spindle assembly and disassembly. Furthermore, results raise the possibility that TPX2 overexpression induces a non-canonical genomic instability, through defective nuclear organization and lamin network reconstitution.

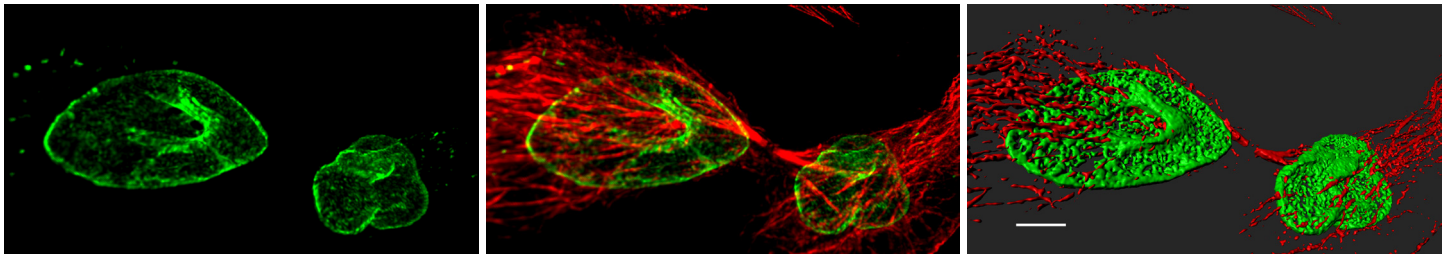

Figure A. TPX2 overexpression interferes with lamin B1 organization at the end of mitosis.
Telophase cell with doughnut-shaped organization of reforming nuclei and MTs passing through the doughnut's hole: lamin B1 staining is shown in green and α-tubulin in red. 3D rendering is shown on the bottom. Scale bar, 5 µm. Images were acquired with the VCS instrument.

Methods

Human hTERT RPE-1 retinal epithelial cells, immortalized with hTERT, were grown on coverslips and processed by immunofluorescence staining for α-tubulin, γ-tubulin, lamin B1, as indicated in figure legends. Cells were counterstained with 4,6-diamidino-2-phenylindole (DAPI) and mounted using ProLong Glass Antifade Mounting (Invitrogen).

Images were acquired through a Nikon Inverted Microscope Eclipse Ti equipped with X-Light V2 spinning disk combined with Video Confocal Super-resolution (VCS; CrestOptics) module based on structured illumination and with a 100x objective (PlanApo Lambda, oil immersion, 1.45 numerical aperture) sectioning the slice in Z with a step size of 0.1 µm.
In order to achieve super-resolution, raw data obtained by the VCS module were processed with a modified version of the joint Richardson-Lucy (jRL) algorithm [33–35], where the out-of-focus contribution of the signal was explicitly added in the image formation model used in the jRL algorithm, and evaluated as a pixel-wise linear "scaled subtraction" of the raw signal. The obtained VCS super-resolved images were elaborated for 3D reconstruction using the NIS-Elements AR 5.20 software (Nikon) and the Imaris 8.1.12 software (Oxford Instruments).

Reference

Excess TPX2 interferes with microtubule disassembly and nuclei reformation at mitotic exit.
Naso, FD., Sterbini, V., Crecca, E., Asteriti, IA., Russo, AD., Giubettini, M., Cundari, E., Lindon, C., Rosa, A., Guarguaglini, G. (2020).
Cells, 9(2). pii: E374. doi: 10.3390/cells9020374.

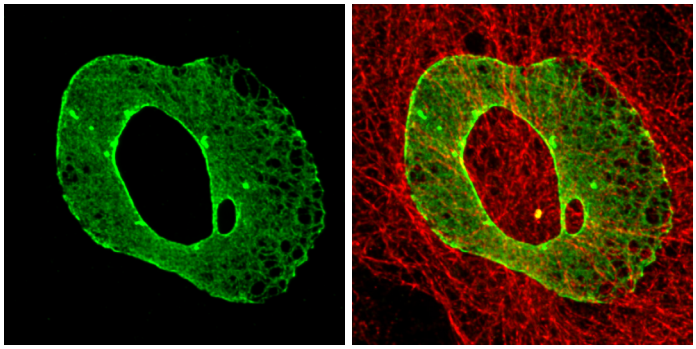

Figure B. TPX2 overexpressing cells display a defective lamin B1 rim in doughnut-shaped nuclei.

The super-resolved image exemplifies the doughnut-shaped nuclei defect with lamin B1 shown in green, α-tubulin in red and γ-tubulin in yellow (all channels merged in the right panel). Scale bar, 5 µm. Images were acquired with the VCS instrument.

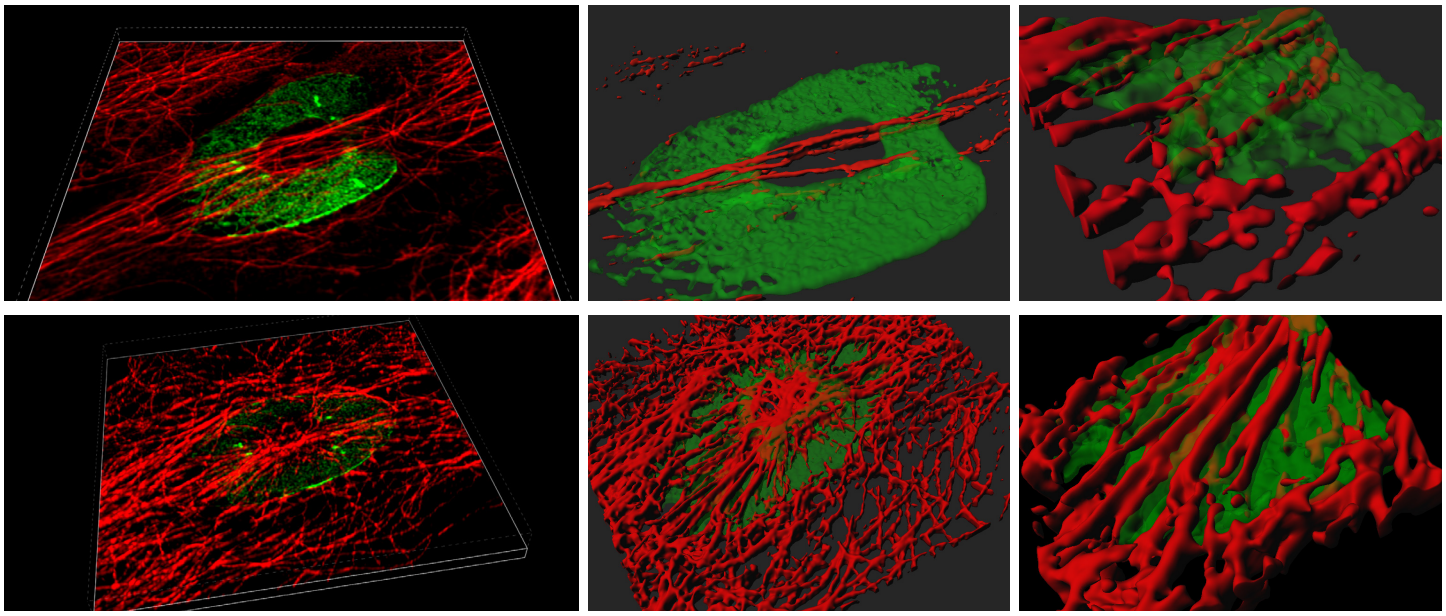

Figure C. Volume and 3D rendering view of unsealed lamina nuclei.

Two examples of doughnut-shaped nuclei with lamin B1 in green, α-tubulin in red: left panels are volume views of super-resolved images; central ones are 3D rendering views, oriented in order to highlight MTs passing through the lamin central hole; right panels, enlargements of specific regions of interest at the nuclear lamina border. Scale bar, 5 µm. Images were acquired with the VCS instrument.