FROM WIDE-FIELD TO SUPER-RESOLUTION FLUORESCENCE MICROSCOPY: DISCOVERY OF HELICAL MICROTUBULE ORGANISATION IN TNT

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Tunneling membrane nanotubes (TNT) are in the focus, as essentials of long-range intercellular communication, for the last 16 years. TNTs are membrane protrusions that connect cytoplasm of initiation and attaching cell and allow the transfer of all kinds of cargo for example organelles, pathogens, proteins and lipids. The structure of TNTs is mainly maintained by the interior cytoskeletal elements. Actin filaments (AFs) and microtubules (MTs) are fundamental components of TNTs, whereas intermediate filaments (IFs) are rarely under investigation. We focused on all representatives of the cytoskeletal elements in normal urothelial (primary cell cultures) and cancer urothelial cells (malignant T24 cell line), both forming TNTs in physiological conditions. We optimized protocol for simultaneous triple labelling of AFs, IFs and MTs and performed wide-field fluorescence microscopy supplemented with deconvolution and super-resolution microscopy with SIM. Wide-field fluorescence microscopy revealed that 59 % of TNTs in normal urothelial cells and 42% of TNTs in cancer cells contained representatives of AFs, IFs and MTs, classifying urothelial TNTs as three-cytoskeletal structures (Figure 1a) [1]. Observation with SIM revealed that MTs twist around IFs in helical organisation (Figure 1b-c). This phenomenon appeared in 4% of TNTs in normal cells and in 11% of TNTs in cancer cells [2].

To sum up, we revealed that urothelial TNTs are in average three-cytoskeletal structures. Moreover, MTs could have unconventional organisation inside TNTs which is helical. We paved the way to reveal how the cytoskeletal composition and organisation correlates with the cell type, TNT longevity and trafficking inside TNTs.

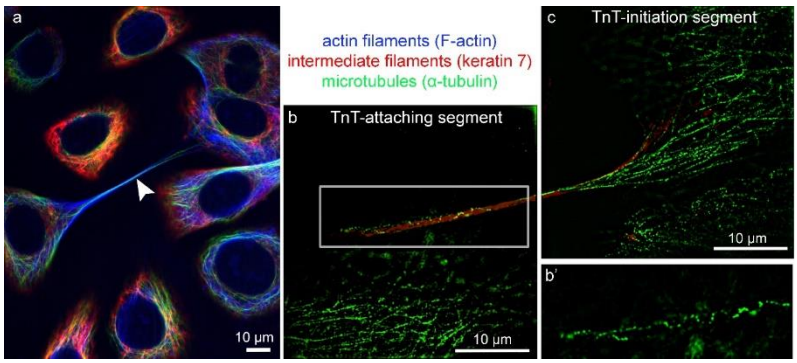

Figure 1: Helical organisation of MTs urothelial TNTs. (a) Presence of AFs, IFs and MTs in TNT (arrowhead). (b, c) MTs enwrap IFs. Helical MT organisation (white box) occurs at TNT-attaching site (b').

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