DETECTION OF MAGNETICALLY MARKED STRUCTURES IN CILIA BY MAGNETIC FORCE MICROSCOPY

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Cilia are microtubule-based cell surface projections with a multitude of functions such as motility, sensory perception and signalling reception and transmission. Generally, they present nine peripheral microtubule (Mts) doublets arranged around two central Mts. They are dynamic and their growth and maintenance are assured by incorporation of tubulin (amongst other proteins) at its distal tip. Structurally known for decades, their importance has increasingly gained relevance in the past few years as several pathologies have been related to cilia. From blindness to polydactyly cilia misfunctions represent a serious health problem, as they are present in the majority of human tissues. Although much progress has been made towards the understanding of cilia structure and assembly, very little is known about its initial growth phase.

Our group has obtained several atomic force microscopy images of growing and fully grown cilia and confirmed the possibility of topographically observe antibodies (anti-cilia proteins) attached to cilia structures.

The scanned surface of normal cilia, apart from the clearly visible microtubule doublets, does not seem to have significant topographical features. However, in immunostained cilia, the surface is rougher and distinct “y”-shaped surface patterns are present, corresponding to bound antibodies. Still, membrane collapse together with inaccessible antibody arms makes it difficult to distinguish every “y”-shape. During these studies a puzzling 3-fold 90-nanometer structure was found at the edge of small growing axonemes. Detailed analysis of this data strongly suggests that, at least in very early stages of cilia growth, axonemes are mainly assembled by pre-mounted material. Interestingly, recent publications also point out new data towards this pre-assembled material hypothesis [1].

Currently, we are developing a new method that encompasses topographic and magnetic information enabling the unequivocal localization of antibodies and thus verify specific protein existence along the cilium, even under the membrane. This is accomplished through the binding of anti-tubulin (for example) antibodies to superparamagnetic particles which are detected by magnetic force microscopy (MFM). Also, it will help confirm our hypothetical pre-assembly findings by providing information about the 3-fold structure’s contents. The MFM data, in relation with topographic information will allow drawing conclusions about the localization of structures containing tubulin (among other proteins).

(Supported by grant FCT/POCI 2010/2004)

References:

[1] Gregory J. Pazour *et al.*, Molecular Biology of the Cell, **Vol. 17** (2006) 3781-3792.