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CRITICAL REVIEW

Ciliary (Dys)function in Human Disease: Mapping the Ciliogenesis Pathway

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The centrosome coordinates formation of the microtubule network, a key component of the mammalian cytoskeleton. Aside from its supporting role in separating genetic material during cell division, the centrosome facilitates the formation of cilia (ciliogenesis) in non-dividing and differentiated cells. Cilia may be motile or non-motile, participating in a range of physiological activities including mucociliary transport, hearing, vision and kidney filtration processes. Ciliopathies occur when cells fail to undergo ciliogenesis or when cilia are improperly formed, affecting all of these functions to varying degrees. While there is increasing emphasis on the genetic factors contributing to these ciliopathies, many proteins involved in the cilia formation program remain elusive. The use of gene knockdown and functional assays are indispensable tools in determining how various proteins contribute to the onset of ciliopathies, ultimately laying the foundation for the development of future gene- and drug-therapy options.

Microfilaments comprise linear polymers of actin proteins that maintain cellular shape. The expansive actin network, which is directly involved in cellular movement, responds to chemical signals released during development, immune events, and other processes pertinent to cell growth and survival.[1] It is well known, for instance, that immune factors stimulate rearrangement of the actin architecture in neutrophils during inflammation, facilitating their amoeboid movement and the elimination of invading pathogens.[2] Microtubules comprise the second distinct class of cytoskeletal filaments that complements the actin network.

Microtubules are composed of alpha- and beta-tubulin heterodimers that polymerize into tubular structures.[3] In mammals and most eukaryotic organisms, microtubule assembly begins at the centrosome, which serves as the cell's microtubule organizing centre (MTOC). As a cytoplasmic organelle, the centrosome is integral to cell division; mitotic microtubules anchor and separate chromosomal DNA, radiating outward from the MTOC across the divisional axis (Figure 1).[3,4] The role of the centrosome is far more robust, however, and extends well beyond the separation of genetic material in mitotic cells. In fact, the centrosome facilitates formation of the cilium, a long slender structure that projects out-

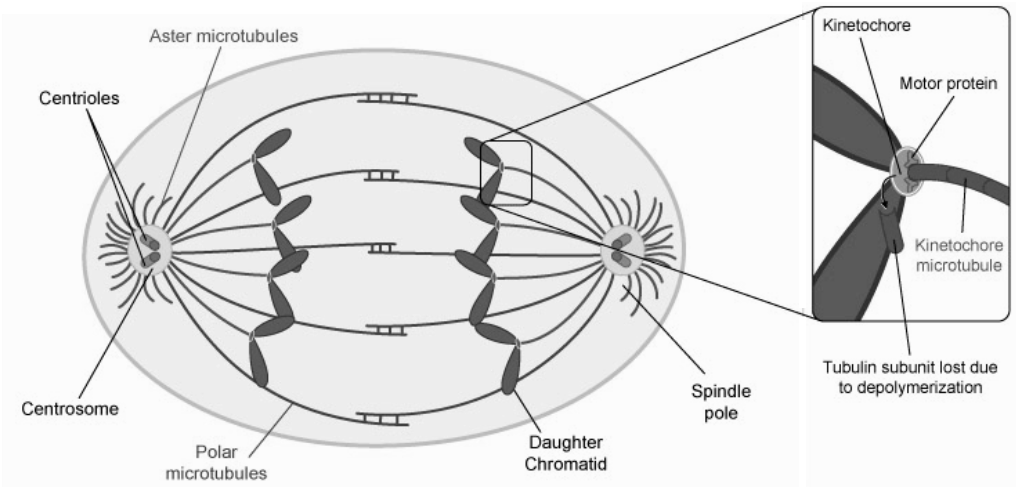

FIGURE 1: Microtubule anchoring during mitosis. Several microtubule sub-types facilitate separation of daughter chromatids during mitosis. Aster microtubules radiate outwards from the MTOC during mitosis and polar microtubules span across opposite poles of the cell. Kinetochore microtubules associate with daughter chromatids, facilitating their separation.[6]

ward from the surface of the cell into the surrounding environment.[5]

CILIA AND HUMAN DISEASE

The physiological roles of cilia have been well studied in humans, and most human cells are now thought to possess cilia. These can range from cells of the fallopian tube, which sweep the egg through the reproductive tract, to tracheal epithelial cells, which sweep debris out of the airways.[7] In large part, cilia are classified according to their function. Those involved in mucociliary transport in the trachea, for instance, are said to be motile since they actively bend and impart force on objects within their vicinity. In contrast, non-motile (primary) cilia function as chemo- and mechano-sensors by transducing external stimuli into a cellular response. Audition, for example, occurs when mechanical sound waves bend hair cells possessing cilia in the inner ear. This electrically excites the hair cells, and stimulates neurons and downstream neuronal networks, culminating in what is perceived as sound.[7] Dysfunction of motile and non-motile cilia has been consistently linked to various developmental and long-term defects, motivating greater research into the genetic basis of cilia formation (ciliogenesis).[8]

Ciliogenesis occurs primarily in non-proliferating (quiescent) and differentiated cells, and is facilitated by the mother centriole of the centrosome. During interphase, the mother centriole migrates to the cell membrane and, through an unknown mechanism, is anchored to the inner cell surface.[6] Here, it polymerizes nine sets of microtubule doublets in a circular arrangement, which forms the cilium's skeleton (Figure 2). In addition, most motile cilia and some primary cilia possess a central set of microtubules doublets, which is needed in order for the cilium to bend as a result of interactions with various microtubule-associated motor proteins, such as dynein.[6,7]

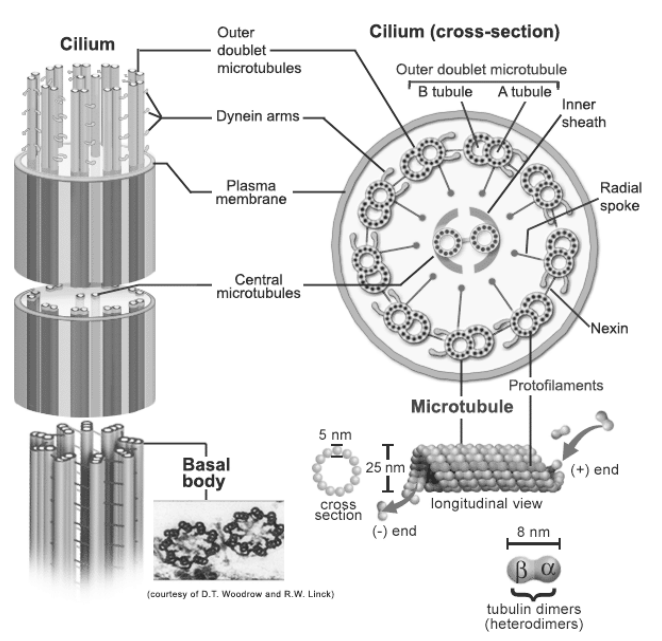

FIGURE 2: Microtubule arrangements in cilia. After migrating to the cell membrane, the mother centriole (indicated as the basal body) facilitates formation of the cilium. The cross-sectional view of the basal body indicates a circular pattern of microtubule triplets arranged along the structure circumference. In contrast, the cilium possesses nine doublets of microtubules. Typically, microtubules of the cilium interact with a host of microtubule-associated proteins like dynein, which is a motor protein. The central doublet of microtubules participates in bending of motile and some primary cilia.[9]

Loss-of-function gene mutations that compromise cilium formation can misdirect growth and transcription factors pertinent to organ development. The left-right asymmetry of the human body plan, for instance, is dependent on the whip-like beating of cilia in the extra-embryonic tissue during early stages of embryogenesis.[12] Without the left-ward sweeping of these growth factors by motile cilia, the heart fails to develop on the mid-left side of the body and the larger lobe of the liver fails to develop on the right.[12]

Until recently, the roles of motile and non-motile cilia in human diseases were poorly characterized and few ciliogenic proteins had been studied. Now, advances in experimental techniques permit scrutiny of the genetic mechanisms that contribute to ciliopathies. Mutations in several genes involved in ciliogenesis have consequently been linked to ciliopathies through genome-wide and population-based studies concerning Senior-Loken, Meckel-Gruber, and Bardedt-Biedl syndromes,[8] which involve auditory, visual and renal system complications, as well as severe developmental defects, such as anencephaly and mental retardation.[9,10] Despite these findings, little is known regarding the mechanism of cilium formation. Before gene therapy or pharmacological interventions can be developed, researchers must therefore elucidate the key players involved in ciliogenesis.

DECODING THE CILIOGENESIS PROGRAM: RECENT ADVANCES AND BREAKTHROUGHS

Much like proteins involved in regulating cell division checkpoints, key players that promote or inhibit ciliogenesis have been identified.[10,11] In a recent study, Tsang et al. identified the mechanism by which centrosomal proteins interact to modulate ciliogenesis in retinal pigmented epithelial cells.[12] Using this cell line, the group demonstrated that interactions between centrosomal protein of 110 kDa (CP110) and centrosomal protein of 290 kDa (Cep290) are necessary in order to suppress cilium formation. Although it was previously known that CP110 participates in centrosome replication, centrosome separation, cytokinesis and ciliogenesis, the exact molecular mechanism(s) by which CP110 modulates these different biological processes were not fully understood. From a clinical standpoint, Cep290 gene mutations have also been extensively linked to various ciliopathies, like nephronophthisis, which is the leading cause of pediatric kidney failure.[13]

Extensive post-translational modifications of centrosomal microtubules allow them to be detected by indirect immunofluorescence techniques, using antibodies directed against the modified tubulin sub-units coupled with fluorophores. Once

exposed to light, protein-bound antibodies reveal the protein's relative position in the cell by re-emitting light at a visible wavelength. In parallel, loss of function mutations associated with ciliopathies can be emulated using silencing RNA (siRNA), a class of double-stranded RNA molecules that interferes with mRNA and protein expression. Importantly, siRNA provides a considerable degree of control over gene-expression and is a valuable tool for studying gene function.

Combining these techniques, Spektor et al. and Tsang et al. found that siRNA-mediated depletion and overexpression of CP110 augmented and suppressed cilia formation, respectively, suggesting that CP110 is a negative regulator of ciliogenesis.[12,15] In striking contrast, Cep290 promotes cilia formation, since siRNA-mediated knockdown of Cep290 leads to inhibition of cilia formation. They also examined protein interaction using co-immunoprecipitation and found that CP110 readily associates with Cep290. Furthermore, unlike wild-type CP110, overexpression of a CP110 mutant incapable of binding Cep290 can no longer inhibit cilia formation in the affected population. Collectively, these findings suggested that Cep290 is inherently ciliogenic and that CP110 is required in order to suppress ciliogenesis.[15]

This experimental approach provided an elegant means of elucidating one component of the cilia formation program. Presently, there are few therapeutic options available for individuals suffering from centrosomal disorders. Undoubtedly, a deeper understanding of the basic science underlying ciliogenesis is critical to the development of drug- and gene-therapy options later on. To these ends, further research is merited in order to elucidate missing stages of the cilia formation pathway, including the identity and properties of the numerous proteins involved in the process. ▪

Reviewed by Dr. William Tsang, PhD

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