GENETIC DISORDERS AFFECTING TUBULIN CYTOSKELETON

Maya D. Markova, Irina V. Chakarova, Ralitsa S. Zhivkova, Venera P. Nikolova, Valentina P. Hadzhinesheva, Stefka M. Delimitreva

Department of Biology, Medical Faculty, Medical University of Sofia, Bulgaria

Summary

The tubulin cytoskeleton is vital for maintenance and dynamics of eukaryotic cells and molecular defects in its components can lead to serious conditions. So far, mutations in genes for alpha-, beta- and gamma-tubulin, motor proteins of the kinesin and dynein family, microtubule-associated and centrosomal proteins have been found to cause disorders in humans. Most phenotypic effects are on the nervous system, leading to abnormal brain development (e.g. lissencephaly and microcephaly) or to neurodegeneration in later life (e.g. amyotrophic lateral sclerosis and frontotemporal dementia). Another group of disorders include the ciliopathies, caused by defects in the axoneme. They include primary ciliary dyskinesia (immotile cilia syndrome), which is characterized by chronic respiratory infections, male infertility and randomly established left-right asymmetry. In most cases, the underlying defects are in axonemal dynein. Mutations in genes for centrosomal components have been shown to cause cortical dysplasia and dwarfism by disrupting the mitotic spindle, and some cases of infertility with maturation arrest are likely to be caused by unidentified mutations damaging the meiotic spindle. In view of these diverse phenotypes, knowledge about mutations affecting tubulin cytoskeleton becomes increasingly useful for clinical practice.

Key words: microtubules, spindle apparatus, oocytes, mutation, infertility

Overview on the tubulin cytoskeleton

The tubulin cytoskeleton, composed of microtubules, is a vital component of eukaryotic cells. It supports the cell shape, maintains the intracellular distribution of organelles, transports cellular components along microtubules and organizes the spindle apparatus responsible for chromosome segregation in dividing cells. Its structural units – the microtubules, are hollow cylinders with a diameter of 25 nm. They are composed of heterodimers of alpha- and beta-tubulin assembling in a polar fashion from the so-called minus end to a growing plus end, a process accompanied by hydrolysis of guanosine

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A third member of the tubulin family, gamma-tubulin, forms a ring complex to which αβ-tubulin dimers join. Structures containing γ-tubulin are called microtubule organizing centers because of their ability to nucleate microtubules by stabilizing the minus end. In animal cells, the main microtubule organizing center is the centrosome, which has a pair of centrioles at its core and pericentriolar material at its periphery. Centrioles also act as basal bodies of cilia and flagella by nucleating their “9 + 2” microtubular axial apparatus called axoneme [2]. Hence, microtubules of animal cells are divided into cytoplasmic and axonemal.

Axonemal microtubules, as well as axonal microtubules of neurons, are stable. However, ordinary cytoplasmic microtubules have an important quality known as dynamic instability: their plus end occasionally switches from slow growth to rapid shrinkage (microtubule catastrophe) and then to growth again (microtubule rescue) [3]. Although these events are random for the individual microtubule, their frequency can be regulated by microtubule-associated proteins [4].

At the onset of cell division, dynamic instability increases dramatically, leading to a rapid collapse of all microtubules that are not stabilized by binding to antiparallel microtubules or chromosome kinetochores. In this way, the interphase microtubular cytoskeleton is transformed into a mitotic or meiotic spindle. The correct spindle structure and attachment of chromosomes to it are vital for the successful outcome of cell division and are controlled by the spindle assembly checkpoint at the metaphase-anaphase transition [1].

The transport function of microtubules is based on their polarity. It requires two opposing types of motor proteins: cytoplasmic dyneins, which drive cargo to the minus ends and kinesins that generally move to the plus ends of microtubules [5]. Axonemal dyneins, related to their cytoplasmic counterparts but forming a distinct subfamily and localizing specifically to the peripheral doublets of the axoneme, mediate the motility of cilia and flagella. They form on the doublet visible protrusions called outer and inner dynein arms (Figure 1A). Using energy of ATP, the dynein arms interact with the adjacent doublet to generate a sliding and, hence, bending force [6]. Genetic disorders affecting cilia are categorized as ciliopathies.

Figure 1. Cross sections of human sperm flagella observed by transmission electron microscopy. A. Normal axoneme. Outer and inner dynein arms are indicated by arrow and asterisk, respectively. Original magnification 59 000x. B. Axoneme from a patient with Kartagener syndrome showing lack of dynein arms. Original magnification 51 000x. Reprinted from [24] © Medical University Pleven

 Disorders due to mutations in tubulin genes

The human tubulin genes form a large family, with a number of loci showing complex tissue-specific expression patterns. Some of them play a crucial role in the central nervous system, particularly for neuronal migration and axonal guidance, i.e. axon outgrowth and maintenance. It should be noted that differentiation and functioning of neurons depends on their axons, which are extremely long and depend on microtubules for support and supply (proteins and most metabolites cannot be synthesized in the axon and must be transported from the cell body using microtubules as “rails”). This is why in recent years, with the development of molecular medicine, more and more disorders affecting the nervous system have been associated with mutations in tubulin genes [7].

The gene TUBA1A encodes a brain-specific isoform of α-tubulin. Its de novo missense mutations in heterozygous state have been found to cause rare disorders of cortical development, particularly lissencephaly 3 [8]. Lissencephaly is characterized by lack of brain folds and grooves as a result of disrupted neuronal migration. The disorder is subclassified into several types caused by mutations in different genes or by environmental factors. Affected children suffer from severe psychomotor retardation, seizures and hypotonia. Many of them remain at the developmental level of a 3-6 month infant for life. Their life span is shortened because of the seizures and the motor impairment predisposing to respiratory infections. The identification of
TUBA1A as a lissencephaly-related gene was facilitated by studies on a mouse model with a mutation in the homologous gene caused by chemical mutagenesis [9].

Another α-tubulin gene, TUBA4A, is described as testis-specific but it is also expressed in the nervous system. It has been recently added to the list of genes which, when mutated, can cause amyotrophic lateral sclerosis. Unlike lissencephaly, which does not allow formation of normal brain structure, amyotrophic lateral sclerosis is a neurodegenerative disorder caused by failure to maintain this structure. Because it primarily affects motor neurons, it is characterized by muscle spasticity, involuntary contractions and atrophy. Heterozygous missense mutations in the TUBA4A gene cause a form of the disease called amyotrophic lateral sclerosis 22 with or without frontotemporal dementia [10]. Unlike lissencephaly, this disorder is often familial, because its late onset allows affected individuals to leave progeny. Studies at cellular level show that the mutant α-tubulin forms small cytoplasmic inclusions in motor neurons. These aggregates are ubiquitinated, i.e. labeled for proteolytic degradation, but apparently cannot be processed efficiently. With regard to microtubule formation, the mutant protein not only shows decreased incorporation into microtubules but also inhibits their assembly and reduces their stability, disrupting the function of the available normal α-tubulin through a dominant-negative mechanism.

Beta-tubulin has also been implicated in human genetic disorders. Mutations in the genes TUBB, TUBB2A and TUBB3 impair neuronal migration during prenatal development and cause complex cortical dysplasia with other brain malformations. Affected individuals have mental retardation, strabismus, axial hypotonia, and spasticity [7]. Mutations in the gene TUBB4A can cause hypomyelinating leukodystrophy [11]. In all mentioned cases, heterozygous mutations exert dominant-negative action as described above for α-tubulin. Actually, the phenotype of patients heterozygous for a TUBB missense mutation was similar to that of a homoyzgous knockout mouse model [7].

Mammalian genome contains two functional genes for γ-tubulin: the ubiquitously expressed TUBG1 and the brain-specific TUBG2. Their function has been studied on homoyzgous knockout mice. Embryos lacking TUBG1 had disorganized mitotic spindles and did not develop beyond blastocyst stage, while the TUBG2 null animals developed and reproduced without any obvious abnormality [12]. In light of these animal data, it is remarkable that heterozygous mutations of the TUBG1 gene have recently been shown to have brain-specific effect in humans. They cause complex cortical dysplasia with other brain malformations, including microcephaly [13]. This is an example of tubulin mutations leading to neurological phenotypes not only in cases of brain-restricted gene expression but also with universally expressed genes, showing again the extraordinarily high requirements to microtubules in the nervous system.

Disorders due to mutations in genes for motor proteins and microtubule-associated proteins

Tubulins are not autonomous in their function. They need microtubule-associated proteins to regulate their organization and motor proteins to mediate microtubule-based transport. It could be expected that mutations affecting some of these proteins will disturb tubulin cytoskeleton as efficiently as mutations in tubulin genes themselves.

Among microtubule-associated proteins, tau is most important for molecular medicine. It is expressed in neurons, where it stabilizes axonal microtubules and promotes their assembly [14]. Homozygous tau knockout mice develop and reproduce normally, but at a later age show symptoms of neurodegeneration, depending to some degree on their strain, i.e. genetic background [15]. In humans, defects in tau cause a whole range of neurodegenerative disorders known as tauopathies [16]. Mutations of the tau gene have so far been shown to cause two disorders: frontotemporal dementia with or without parkinsonism and progressive supranuclear palsy. Other tauopathies are due to disturbances of tau phosphorylation, most likely caused by mutations in other genes [17].

Among microtubule-associated motor proteins, kinesin KIF11 has been implicated in human disease. (Kinesins are designated by KIF, from “kinesin family member”, and a number). KIF11 is a plus end-directed microtubule motor that cross-links microtubules and mediates their antiparallel sliding during formation of mitotic spindle. It also increases efficiency of translation by attaching ribosomes to microtubules [18].

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Homozygous KIF11 knockout mice die before reaching blastocyst stage. In humans, heterozygous KIF11 de novo mutations cause microcephaly with or without bilateral chorioretinopathy, lymphedema, or mental retardation [19].

The antagonists of kinesins in the cell are dyneins that act as minus end-directed microtubule motors. A member of their family, cytoplasmic dynein 1 heavy chain 1 (DYNC1H1), mediates various forms of intracellular motility, including retrograde axonal transport and formation of mitotic spindle in prophase. Dominant mutations of its gene impair neuronal migration and cause axonal Charcot-Marie-Tooth disease, mental retardation or spinal muscular atrophy [13].

A related gene, cytoplasmic dynein 2 heavy chain 1 (DYNC2H1), is expressed in a number of non-ciliated tissues but is also important for intra-flagellar transport in ciliated epithelia. Its mutations have been found to cause a chondrodysplasia called short-rib thoracic dysplasia 3 with or without polydactyly. This term unifies too disorders: Jeune asphyxiating thoracic dystrophy characterized by shortened ribs and long bones and accompanied in some cases by polydactyly, and the even more severe short-rib polydactyly syndrome type III characterized by variable malformations, early prenatal manifestation and lethality [20, 21]. As we see, unlike the disorders described above, short-rib thoracic dysplasia 3 affects the skeleton rather than the nervous system. The mechanism is presumably based on the role of primary cilia in transduction of hedgehog pathway signals that are crucial for skeletal development; in other words, this disorder is a skeletal ciliopathy [22].

Similarly to the skeletal ciliopathy due to DYNC2H1 mutation (described above), primary ciliary dysgenesis is an autosomal recessive disorder. Mutations causing this disorder have so far been found in 29 genes and their number continues to grow [26]. The disorder types caused by mutations in individual genes are designated by numbers. The first ten of them are listed in Table 1. These genetic data in accordance with the ultrastructural observations show that primary ciliary dyskinesia is mostly due to defects of axonemal dyneins.

Among γ-tubulin-associated proteins, mutations causing genetic disorders have been described for pericentrin. Its function is to anchor γ-tubulin ring complexes to the salt-insoluble centrosomal core. In humans, absence of functional pericentrin leads to microcephalic osteodysplastic primordial dwarfism type II. The patients have an average adult height of 100 cm and a brain size comparable to that of a 3-month old infant, though intelligence is near-normal. At cellular level, disorganization of mitotic spindles and missegregation of chromosomes is observed. This phenotype is caused by major mutations (nonsense or frameshift) in homozygous or compound heterozygous state [27].

Disorders due to unidentified mutations

While the clinical presentation of the above mentioned disorders has been studied in detail for a long time, many of the underlying mutations were unknown only 10 years ago. Despite the fast advances of molecular medicine, there are still a
Table 1. Some types of primary ciliary dyskinesia (abbreviated as CILD), listed according to the Online Mendelian Inheritance in Man database

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Mutated gene</th>
<th>Affected protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>CILD1</td>
<td>DNAI1</td>
<td>Axonemal dynein intermediate chain 1</td>
</tr>
<tr>
<td>CILD2</td>
<td>DNAAAF3</td>
<td>Axonemal dynein assembly factor 3</td>
</tr>
<tr>
<td>CILD3</td>
<td>DNAH5</td>
<td>Axonemal dynein heavy chain 5</td>
</tr>
<tr>
<td>CILD4</td>
<td>Unidentified gene on chromosome 15</td>
<td></td>
</tr>
<tr>
<td>CILD5</td>
<td>HYDIN</td>
<td>Homolog of mouse hydrocephalus-inducing protein</td>
</tr>
<tr>
<td>CILD6</td>
<td>TXNDC3</td>
<td>Thioredoxin domain-containing protein 3</td>
</tr>
<tr>
<td>CILD7</td>
<td>DNAH11</td>
<td>Axonemal dynein heavy chain 11</td>
</tr>
<tr>
<td>CILD8</td>
<td>Unidentified gene on chromosome 15</td>
<td></td>
</tr>
<tr>
<td>CILD9</td>
<td>DNAI2</td>
<td>Axonemal dynein intermediate chain 2</td>
</tr>
<tr>
<td>CILD10</td>
<td>DNAAF2</td>
<td>Axonemal dynein assembly factor 2</td>
</tr>
</tbody>
</table>

lot of conditions that are probably caused by genetic defects in tubulin cytoskeleton but the affected genes have not yet been identified.

As shown above by the examples of TUBG1, KIF11, DYNC1H1 and pericentrin, different mutations may affect mitosis by disrupting the spindle apparatus. It could be expected that some mutations would affect specifically the meiotic spindle, resulting in maturation arrest and/or high frequency of gamete aneuploidy. Maturation arrest of all germ cells at the same stage of meiosis has been described both for spermatocytes of infertile men [28] and for oocytes of women undergoing in vitro fertilization [29]. It is very likely that mutations in genes needed for meiotic spindle formation underlie some cases of infertility. However, due to the strong influences of age, hormonal and environmental factors on human meiosis and the methodological difficulties inherent in such studies, the genetic cause still remains just a hypothesis and no candidate genes have yet been identified. A case report has described infertility in the offspring of a consanguineous marriage: two sisters with uniform metaphase I arrest of oocytes and two brothers with unknown cause. Based on these observations, a genetic factor with autosomal recessive inheritance has been suggested [30].

Meiosis in oocytes is likely to be more sensitive to spindle defects than in spermatocytes, due to the long periods of arrest and the centrosome reduction during oogenesis. This reduction, which has the function to prevent parthenogenesis, leads to an acentrosomal barrel-shaped spindle unique for animal cells (Figure 2). An approach to assess the influence of genetic factors on the oocyte spindle apparatus is to compare different mouse strains. We have studied oocyte meiotic maturation in two inbred strains (BALB/c and C57/Black) and evaluated their spindle defects – too large or too small size of the spindle, large poles, abnormal number of poles, additional spindles, disoriented fibers or overall degradation of the spindle. The two strains differed both in their maturation rates (i.e. proportion of oocytes successfully reaching metaphase II) and in the characteristic spectrum of defects, indicating influence of genetic factors. F1 hybrids in many respects showed results superior to those of both parent strains but had a tendency to resemble the mother strain, suggesting the possibility of genomic imprinting [31].

Figure 2. Immunofluorescent image of a mature mouse oocyte. Tubulin is labeled in green and DNA in blue. The barrel-shaped spindle of meiotic metaphase II is indicated by MII and the first polar body by PB. Original magnification 400x. Reprinted from [31]

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Conclusions

In recent years, mutations in genes encoding tubulins, microtubule-associated and motor proteins have been shown to cause a range of conditions including disorders of brain development, neurodegenerative disorders, chondrodysplasia, dwarfism and primary ciliary dyskinesia. There are also indirect data that such mutations could disturb meiosis, particularly oocyte meiosis. With the development of molecular medicine, more genetic defects of the tubulin cytoskeleton will undoubtedly be discovered. While they are presumed to be rare, their true prevalence is unknown, especially for milder phenotypes. Therefore, knowledge about the mutations affecting microtubules and the diverse spectrum of their possible effects is becoming increasingly useful for clinical practice.

Acknowledgements

This work was supported by Medical University of Sofia Grant No. 29/2014. Mol Biotechnol. 2009 Oct;43(2):177-91.

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