# Consequences of Numerical Centrosome Defects in Development and Disease

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**Abstract**

Defects in centrosome number or structure can have considerable consequences for the physiology of an organism. Aberrant centrosome number has been proposed for a century to contribute to genome instability and tumour formation. However, in the last decade, mutations in centrosome genes have been described in diseases characterised by defective growth. Centrosome dysfunction can therefore have opposite effects on the homeostasis of the organism. Here we discuss how deregulation of centrosome number during embryonic development might contribute to growth defective syndromes such as autosomal recessive primary microcephaly (MCPH) and primordial dwarfism. We further discuss how the same defects might play a role in cancer when present in adult tissues.

## 5.1 Introduction

The centrosome is the major microtubule-organising centre of animal cells (Kellogg et al. 1994). It participates in different processes such as cell division, motility and polarity, mainly by organising the microtubule network. Centrosomes are not present in plants, whereas fungi have an analogous structure called the spindle pole body (Marshall 2009).

The centrosome is composed by two centrioles surrounded by the pericentriolar material (PCM) (Nigg and Raff 2009) (for a discussion of PCM structure and function, see also the Chap. 3 by Comartin and Pelletier). Centrioles are cylindrical structures made of nine microtubule triplets arranged in a ninefold symmetry. They recruit and organise a large number of proteins forming the PCM (Bobinnec et al. 1998).

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Centriole number is tightly regulated. For most part of the cell cycle, the two centrioles are linked and placed orthogonally to each other. This configuration is called centriole engagement, and a new centriole is not formed as long as the pre-existing centrioles are engaged (Tsou and Stearns 2006). Centriole disengagement takes place usually at the end of mitosis when the daughter cells inherit one centrosome with two separated centrioles (Kuriyama and Borisy 1981). Centriole disengagement is thought to be the licence to allow centriole duplication (Tsou and Stearns 2006), which occurs only once per cell cycle.

Five proteins, ZYG-1, SPD-2, SAS-4, SAS-5 and SAS-6, were identified in *Caenorhabditis elegans* as essential for centriole biogenesis (Dammermann et al. 2004; Delattre et al. 2004; Kemp et al. 2004; Kirkham et al. 2003; Leidel et al. 2005; Leidel and Gonczy 2003; O'Connell et al. 2001; Pelletier et al. 2004). These proteins are recruited in a precise temporal order (Delattre et al. 2006; Pelletier et al. 2006). SPD-2 is the first to be recruited to the parental centriole, which in turn is required for ZYG-1 recruitment. Shortly after, a complex conteining SAS-5 and SAS-6 is recruited to structurally form the centriole. Finally SAS-4 is recruited to allow the incorporation of microtubules into the centriole wall.

Remarkably, all these genes have a sequence or a functional ortholog in most animals (Carvalho-Santos et al. 2010; Hodges et al. 2010). Human PLK4, also called SAK in *Drosophila*, is the functional ortholog of ZYG-1 and, like ZYG-1, is a serine-threonine kinase (Bettencourt-Dias et al. 2005; Habedanck et al. 2005) (Fig. 5.1). CEP192 is the human ortholog of SPD-2 (Andersen et al. 2003; Pelletier et al. 2004). SAS-4 orthologs are called CPAP in humans (Tang et al. 2009) and DSas-4 in *Drosophila* (Basto et al. 2006). STIL and Ana2 are, respectively, the human and the *Drosophila* functional orthologs of SAS-5 (Arquint et al. 2012; Stevens et al. 2010; Tang et al. 2011; Vulprecht et al. 2012). SAS-6 is called HsSAS-6 in humans and DSas-6 in flies (Gopalakrishnan et al. 2010; Leidel et al. 2005; Peel et al. 2007; Rodrigues-Martins et al. 2007a; Strnad et al. 2007).

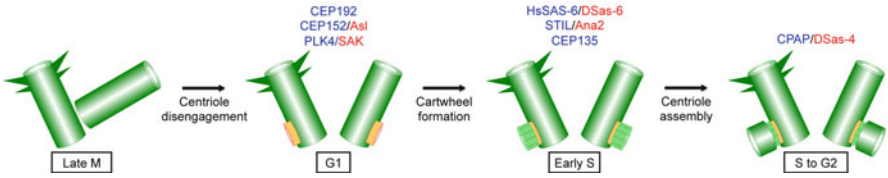


**Fig. 5.1** The centriole duplication cycle. For most part of the cell cycle, the two centrioles (*dark green cylinders*) are linked and placed orthogonally to each other. This configuration is called centriole engagement. At the end of mitosis, each daughter cell inherits one centrosome with two separated centrioles (centriole disengagement). This event is permissive to centriole duplication. Centriole duplication is initiated when CEP192 and CEP152/Asl recruit PLK4/SAK at the proximal end of the mother centriole (*yellow ring*). PLK4/SAK activity is required for cartwheel formation (*light green ring*). The cartwheel is composed of SAS-6, STIL/Ana2 and CEP135 and is the first visible structure of the new centriole. Subsequently, SAS-4 is recruited to allow the incorporation of nine sets of microtubules (centriole assembly). During G2 the procentriole (*short dark green cylinders* at the proximal end of the mother centriole) elongates to achieve the size of the mother

Similarly to their orthologs in *C. elegans*, centriolar recruitment of these proteins is hierarchically regulated (Kleylein-Sohn et al. 2007). PLK4/SAK is the master regulator of centriole duplication, and in human cells, it is recruited to the mother centriole by CEP192 in collaboration with another protein not present in *C. elegans* called CEP152 (Kim et al. 2013; Sonnen et al. 2013). In *Drosophila*, SPD-2 is dispensable for centriole duplication (Dix and Raff 2007; Giansanti et al. 2008) and SAK recruitment is entirely fulfilled by the CEP152 ortholog, asterless (Asl) (Dzhindzhev et al. 2010). PLK4/SAK triggers centriole formation through direct interaction with STIL/Ana2, and its phosphorylation by PLK4/SAK (Dzhindzhev et al. 2014; Kratz et al. 2015; Ohta et al. 2014) is then required for centriolar loading of HsSAS-6/DSas-6. STIL/Ana2 and HsSAS-6/DSas-6 form the cartwheel, which is the first identifiable structure in pro-centriole assembly (Guichard et al. 2010; Kitagawa et al. 2011b; Kuriyama 2009; van Breugel et al. 2011). Subsequently, CPAP is recruited and, according to cell type, nine sets of microtubule (Kleylein-Sohn et al. 2007) singlets, doublets or triplets will complete the centriole structure (Fig. 5.1). Although in humans extra proteins participate in procentriole assembly, such as CEP135, CP110 and γ-tubulin (Kleylein-Sohn et al. 2007), the core duplication machinery is well conserved through evolution (Carvalho-Santos et al. 2010; Hodges et al. 2010) (see Fig. 5.1 and Chap. 3 for further details on centriole duplication).

## 5.2 Animals Without Centrosomes

In 1887, Van Beneden and Boveri described the centrosome as "the organ for cell division". This statement was justified by the presence of centrosomes at the spindle poles, suggesting a function in spindle formation. For this reason, it has long been accepted that centrosomes were essential for cell division. However, the discovery that many eukaryotic organisms, such as plants, do not have centrosomes suggested that probably centrosomes are dispensable to form a bipolar spindle, at least in some cell types. Indeed, many cells form a bipolar spindle and divide even in absence of centrosomes. One interesting example is the mouse embryo that forms "acentrosomal" spindles in the first cleavages (Szollosi et al. 1972). Other examples are female oocytes from most animal species that assemble "acentrosomal" spindles during both meiotic divisions (Schatten 1994). In the absence of centrosomes, microtubules are nucleated at the vicinity of the chromatin, and with the help of molecular motors, spindle poles become focused (Heald et al. 1996; Karsenti et al. 1984; Khodjakov et al. 2003; Maiato et al. 2004). In addition to this pathway, microtubules can also be generated within the mitotic spindle from pre-existing microtubules. In this case, γ-tubulin is required and its localisation depends on the augmin complex (Goshima et al. 2008).

All these pathways exist in cells that form mitotic spindle via the "classical" centrosome-dependent mechanism, and the three collaborate to assemble a functional bipolar spindle that accurately segregates chromosomes (Meunier and Vernos 2012).

Even if some cell types in animals can undergo cell division in the absence of centrosomes, it was difficult to imagine that an entire organism could develop without centrosomes. In a surprising study, it was shown that the planarian *Schmidtea mediterranea* does not contain centrosomes at any stage of its life cycle (Azimzadeh et al. 2012). Interestingly, planarians need to assemble cilia in some cell types, and *de novo* centriole formation in these cells ensures the presence of basal bodies for ciliogenesis. PCM proteins such as SPD-2/CEP192 and Cnn/CDK5RAP2 are absent from *S. mediterranea,* and cell division does not depend on centrosomes. Since centrosomes also play important roles in spindle positioning during oriented cell divisions (Morin and Bellaiche 2011), these results also put in evidence that regeneration, a process frequently used in planaria, does not require centrosome-dependent spindle positioning (Cardona et al. 2006).

But what happens if centrosomes are removed from somatic cells that normally contain centrosomes? This question was initially addressed in vertebrate cells where centrosomes were removed by laser ablation or microsurgery (Hinchcliffe et al. 2001; Khodjakov et al. 2000). In these cells, a bipolar spindle, which could correctly segregate chromosomes within the following cell division, was assembled. Recently however, it has been shown that permanent removal of centrosomes in chicken DT40 cells by knockout of either *CEP152* or *STIL* results in abnormal chromosome segregation (Sir et al. 2013). In these cells, the spindle was disorganised explaining probably the high rate of segregation errors observed. The authors proposed that organisms with high number of chromosomes, such as chicken, which has 78 chromosomes, strongly rely on centrosome-driven spindle assembly for mitotic fidelity. It is important to mention, however, that these cells are non-adherent and might require centrosomes for mitotic spindle assembly while other cell types that undergo mitosis while adhering to a substrate do not (Hinchcliffe et al. 2001; Khodjakov et al. 2000).

Flies that carry mutations in the centriole duplication genes *asl*, *DSas-4* and *PLK4*/*SAK* undergo larval development giving rise to adults without any morphological defect (Basto et al. 2006; Bettencourt-Dias et al. 2005; Blachon et al. 2008). Importantly, this is possible because maternally provided components ensure centriole duplication at early developmental stages. In the absence of centrosomes, early embryonic development is impaired and embryos arrest during syncytial stages (Stevens et al. 2007). The centrosome-dependent spindle assembly mechanism is probably extremely important during the rapid mitotic cycles occurring after fertilisation, and astral microtubules are required for nuclear separation after anaphase in the preblastoderm cytoplasm (Telley et al. 2012).

Acentriolar flies also lack cilia and flagella, which are essential in *Drosophila* for adult viability and male fertility, respectively. In flies very few cells contain cilia. Type I mechanosensory neurons are ciliated, and adults that lack centrioles are severely uncoordinated and die a few hours after eclosion (Baker et al. 2004; Dubruille et al. 2002; Gogendeau and Basto 2010; Martinez-Campos et al. 2004). Another cell type that requires centrioles for cell division in flies are primary spermatocytes that normally have long centrioles containing microtubule triplets. In the absence of centrioles, meiotic spindles present broad poles and are highly

disorganised giving rise to unviable aneuploid progeny (Basto et al. 2006; Bettencourt-Dias et al. 2005; Martinez-Campos et al. 2004; Rodrigues-Martins et al. 2007b).

Centrosomes, through astral microtubule nucleation, also contribute to accurate spindle positioning. This is particularly important when cell fate determinants should be segregated into daughter cells during asymmetric and even symmetric divisions (Knoblich 2008). *Drosophila* acentriolar larval brain stem cells (NSCs) divide symmetrically and give rise to two stem cells, at the expense of differentiating cells (Basto et al. 2006; Giansanti et al. 2001; Megraw et al. 2001), which causes tumour formation in transplantation assays (Castellanos et al. 2008). Centrosomes also participate in spindle positioning in *Drosophila* male germline stem cells (Yamashita et al. 2003); however, other mechanisms contribute to tissue homeostasis in the absence of centrosomes (Riparbelli and Callaini 2011; Sheng and Matunis 2011). In female germline stem cells however, centrosomes are dispensable and spindle positioning depends on the function of the fusome (Stevens et al. 2007), a membrane skeleton-enriched structure typical of the insect germline (Lin et al. 1994).

In vertebrates, most cells contain a primary cilium, and centriole loss has severe consequences during development. Embryos lacking cilia have defective body plan organisation caused by disruption of hedgehog signalling pathway and arrest at E10.5–11.5 (Goetz and Anderson 2010). Recently, the developmental functions of mammalian centrioles *in vivo* have been analysed (Bazzi and Anderson 2014). Characterisation of a null *Cpap* (the *SAS-4* ortholog) mutant mouse revealed that centrosomes are essential during embryonic development. $Cpap^{-/-}$ mice died at early stages (E9.0) with increased p53-dependent cell death. Interestingly, increase in DNA damage or aneuploidy was not observed. Instead, cells displayed a prolonged prometaphase, and consequently mitosis completion was delayed, suggesting a requirement for centrosomes for rapid bipolar spindle assembly in vertebrates. Null mutant mice for other centriole duplication genes, *Plk4*, *Stil* and *Cep152*, also arrested early in development, at the same stage of the $Cpap^{-/-}$ mice, showing increased apoptosis and increased p53 levels (Bazzi and Anderson 2014; Hudson et al. 2001; Izraeli et al. 1999). Intriguingly, Bazzi and colleagues noticed that in embryos lacking centrioles, regions with higher proliferation rates showed higher p53 levels. Since these embryos died earlier than those lacking cilia (Huangfu et al. 2003), it is possible that the absence of centrioles *per se* up-regulates p53 in rapidly proliferating cells, causing widespread cell death and consequent lethality. Probably, the apoptotic pathway is triggered in cells that are not able to go through mitosis as fast as they should. In zebra fish, depletion of *stil* induced a similar phenotype (Pfaff et al. 2007). Apoptosis was also increased and embryos died between 7 and 10 days post-fertilisation. Spindles were monopolar or highly disorganised, which resulted in delayed mitotic progression.

Overall, these studies show an unexpected up-regulation of cell death by apoptosis in response to prolonged mitosis or mitotic arrest. It will be important in the future to determine why the apoptotic pathway is triggered in the absence of centrosomes, when aneuploidy is not being generated (Bazzi and Anderson

2014). In particular it will be essential to understand how the lack of centrosomes is detected in embryonic cells and then translated into p53 up-regulation and apoptosis.

## 5.3 Animals with Extra Centrosomes

Differentiated cells have one centrosome that organises the microtubule network or cilia, whereas cycling cells, in mitosis, have two centrosomes to form a bipolar spindle. The presence of more than two centrosomes in a cell is called centrosome amplification, and it has been described in both physiological and pathological conditions. There are three main ways to induce centrosome amplification:

1. Cytokinesis failure: it generates tetraploid cells with four centrosomes. Cytokinesis failure occurs physiologically in certain mammalian tissues during postnatal growth such as hepatocytes (Guidotti et al. 2003).
2. Cell fusion: during development, cell fusion is involved in many processes, such as the formation of trophoblast cells in the mammalian placenta, muscles and osteoclasts (Oren-Suissa and Podbilewicz 2007). Cell fusion can also be involved in wound healing as shown in *Drosophila* larval and adult epidermis (Galko and Krasnow 2004; Losick et al. 2013).
3. Centrosome cycle deregulation: the levels of proteins involved in centriole duplication are highly regulated during the cell cycle (Marthiens et al. 2012). Their overexpression can trigger centrosome amplification (Kleylein-Sohn et al. 2007). For example, when *PLK4* is overexpressed, multiple centrioles are formed in S phase in the typical rosette-like structure surrounding the mother centriole (Habedanck et al. 2005; Kleylein-Sohn et al. 2007). At the end of mitosis, they disengage and duplicate to give rise to extra centrosomes.

The presence of extra centrosomes can induce multipolar divisions, which are in most cases not viable (Ganem et al. 2009). Cells evolved several mechanisms to enable bipolar division in the presence of centrosome amplification, and the predominant mean is the clustering of extra centrosomes in two main spindle poles (Basto et al. 2008; Kwon et al. 2008; Leber et al. 2010; Marthiens et al. 2012; Quintyne et al. 2005; Ring et al. 1982). Clustering is achieved mainly by combining spindle-intrinsic microtubule binding forces and actin-regulating forces at the cell cortex (Kwon et al. 2008). However, this mechanism can hide a threat. Extra centrosomes induce the formation of multipolar spindle intermediates during prometaphase, which promote merotelic attachments (one kinetochore attached to microtubules nucleated by different poles) and consequent chromosome missegregation during anaphase (Ganem et al. 2009; Silkworth and Cimini 2012). In certain cell types, extra centrosomes favour the nucleation of extra cilia, which leads to dilution of cilia signalling molecules such as members of the sonic hedgehog signalling pathway (Mahjoub and Stearns 2012).

Consequences of centrosome amplification at the level of the whole organism have been studied only in *Drosophila* so far. When *SAK* (the *PLK4 Drosophila* ortholog) is overexpressed in all the cells of the body, almost 60 % of somatic cells present centrosome amplification. Embryonic development is highly compromised, as clustering mechanisms are not efficient during early development (Basto et al. 2008). Larval development is slightly delayed, but adults are morphologically normal, viable and fertile. Neuroblasts (NBs) of the larval brain, which are neural stem cells, always divide in a bipolar fashion even though they harbour extra centrosomes, thanks to the very efficient centrosome clustering and inactivation mechanisms (lack of microtubule-nucleating capacity). However, in certain cases, spindles are mispositioned which results in defects in asymmetric cell division and in the generation of extra stem cells at the expense of differentiating cells. As a consequence, in transplantation assays, these brains can over-proliferate and induce tumours that kill the host prematurely (Basto et al. 2008) (see also next paragraphs). In the wing imaginal disc, the larval epithelium that gives rise to the adult wings, mechanisms of centrosome clustering and centrosome inactivation are also present but not fully efficient (Sabino et al. 2015). As a result, tripolar divisions and chromosome segregation defects occur with only minor defects in spindle positioning. This leads to aneuploid cells able to proliferate and induce tumorigenesis when transplanted into WT hosts. Hence, these studies show that centrosome amplification, at least in *Drosophila*, is a tumour-initiating event (Basto et al. 2008; Sabino et al. 2015).

The consequences of centrosome amplification in vertebrates are still not known with the exception of centrosome amplification in the mouse central nervous system (CNS) (Marthiens et al. 2013). Contrary to flies, the presence of extra centrosomes in mouse neural stem cells does not perturb spindle orientation. However, it causes aneuploidy and consequent cell death due to inefficient clustering. A major consequence of centrosome amplification in the mouse CNS is a severe reduction in brain size, a condition also known as microcephaly (see below) (Fig. 5.2).

## 5.4 Centrosomes and Disease

### 5.4.1 Centrosome Defects and Growth Failure

Generally, the number and size of cells define the size of organs and organisms (Conlon and Raff 1999). The balance between cell proliferation, differentiation and cell death contributes to determine the number of cells at the end of development (Conlon and Raff 1999). Changes in one of these parameters, in particular during embryonic development when body size is being established, can lead to growth defects (Klingseisen and Jackson 2011).

Progenitor cells undergo two different types of division: (1) to enlarge the pool of progenitors, they divide symmetrically forming two identical cells and (2) to allow differentiation, they divide asymmetrically giving rise to one progenitor cell and to another, generally more committed that will ultimately differentiate

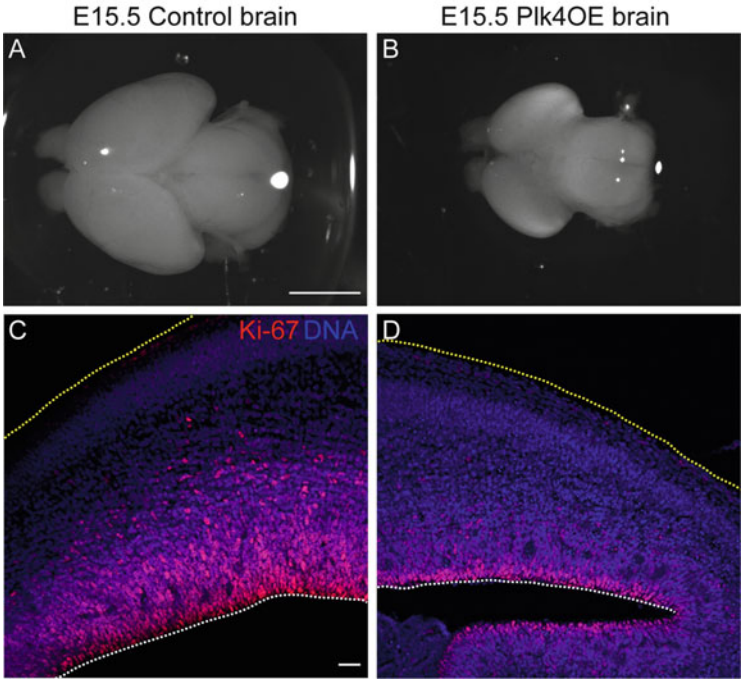


**Fig. 5.2** Consequences of *Plk4* overexpression (*Plk4OE*) in the developing mouse brain. Centrosome amplification in mouse embryonic neural stem cells (NSCs) caused by the overexpression of *Plk4* results in microcephaly. Dorsal views of control (**a**) and Plk4OE (**b**) brains at E15.5. Scale bar = 2 mm. Control (**c**) and Plk4OE (**d**) E15.5 brain sections immunostained for the cycling proliferating marker Ki67 (red). DNA is shown in blue. Scale bar = 12 μm

(Tajbakhsh et al. 2009). Accurate timely control of the switch from symmetric to asymmetric division is essential to guarantee correct development and body size.

Although the mechanisms behind growth retardation during embryonic development are not entirely known, mutations in genes encoding for centrosome and centrosome-associated proteins have been reported in diseases characterised by growth deregulation, such as autosomal recessive primary microcephaly (MCPH) and the primordial dwarfism diseases Seckel syndrome (SCKS) and microcephalic osteodysplastic primordial dwarfism type II (MOPD-II).

The common feature of these syndromes is microcephaly, which is clinically defined as a smaller brain than the mean for sex, age and ethnicity, with the occipitofrontal head circumference (OFC) equal or less than -2 standard deviation (SD) (Roberts et al. 2002). In MCPH, SCKS and MOPD-II, brain structures are proportionated and present minor malformations. The phenotype of MCPH and SCKS is similar but more severe in the latter (Klingseisen and Jackson 2011). In SCKS, effects in intrauterine and postnatal growth are more pronounced. The mean OFC in children and adults is around -9 SD, whereas mean height is -7 SD. They usually suffer of mental retardation and a characteristic appearance with a narrow

and sloping forehead, prominent eyes, large and convex nose and small jaw (Hall et al. 2004; Majewski and Goecke 1982).

Stature measurement is usually the parameter used to distinguish between MCPH and SCKS: patients with normal height or between -1 SD and -2 SD are classified as MCPH, whereas those between -4 SD and -12 SD as SCKS (Verloes et al. 1993). Compared to MCPH and SCKS, in MOPD-II, growth retardation is more severe and accompanied by highly proportional reduced body size (Majewski et al. 1982). Primordial dwarfisms and MCPH are extremely rare autosomal recessive one-gene disorders, with higher incidence in populations where consanguineous marriages are common (Woods et al. 2005). MCPH, for example, has been reported in only about 100 families worldwide (Kaindl et al. 2010).

Non-centrosomal genes can also lead to microcephalic primordial dwarfisms, usually accompanied with other malformations. Meier-Gorlin syndrome is characterised by small ears, absent/hypoplastic patellae and short stature (Gorlin et al. 1975). Taybi-Linder syndrome (also known as MOPD type I or III) has profound growth retardation and severe brain malformation of the cerebral cortex (Sigaudy et al. 1998). In patients affected by lissencephaly, the brain is smooth with a thickened cortex, although microcephaly is not always present (Dobyns et al. 1993).

Since the majority of genes found mutated in microcephaly are centrosome or spindle pole-associated genes, we will focus this chapter on these, while mentioning briefly other non-centrosomal genes.

#### 5.4.1.1 Etiology of Microcephaly

So far, three main causes have been proposed to be at the basis of microcephaly. They ultimately converge at the same outcome, depletion of the pool of progenitors during brain development, and consequently fewer cells can form the brain. These three causes are DNA damage response, spindle orientation and spindle integrity (Fig. 5.3).

The first MCPH gene identified, *microcephalin,* has been associated with the DNA damage checkpoint (Jackson et al. 1998). The DNA damage checkpoint maintains cells blocked in G2 through the activation of the G2/M checkpoint, inhibiting entry into mitosis to allow DNA repair. Defects in DNA repair result in apoptosis or premature differentiation, at least in certain cell types (Inomata et al. 2009; Schneider et al. 2013; Sherman et al. 2011).

Defects in spindle orientation have been proposed to result from mutations in at least three MCPH genes, *Aspm*, *CDK5RAP2* (also known as *Cep215*) and *CPAP* (also called *CenpJ*) (Fish et al. 2006; Kitagawa et al. 2011a; Lancaster et al. 2013; Lizarraga et al. 2010). In both symmetric and asymmetric dividing cells, spindle orientation determines the plane of cell division and consequently the correct segregation of cell fate determinants (Morin and Bellaiche 2011). For example, *Aspm* knockdown in the mouse neuroepithelium caused defects in spindle orientation that led to premature differentiation during neurogenesis (Fish et al. 2006).

Certain MCPH mutations cause disruption of centrosome integrity and numerical defects, affecting also spindle formation. Among these, mutations in the master

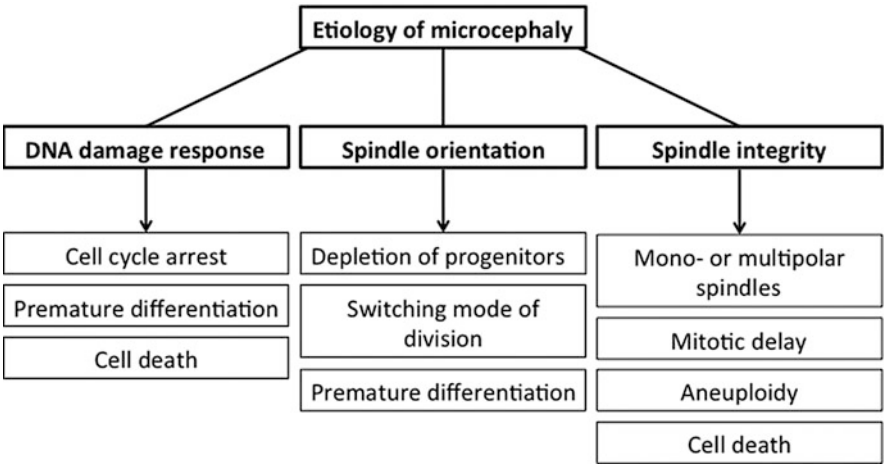


**Fig. 5.3** Etiology of microcephaly. Three major causes of microcephaly have been proposed so far: (i) DNA damage response, (ii) spindle orientation, (iii) spindle integrity. Through different means, they all lead to depletion of the pool of progenitors

regulator of centriole duplication *PLK4* have been recently described (Martin et al. 2014; Shaheen et al. 2014). Patient-derived fibroblasts showed reduced protein levels and consequent centriole duplication failure. Mitotic spindle formation was also affected with a predominance of monopolar spindles (Martin et al. 2014).

Another type of centrosome dysfunction that also impacts in brain size is centrosome amplification (Marthiens et al. 2013). Overexpression of *Plk4* in embryonic NSCs resulted in the presence of supernumerary centrosomes in one third of the NSC population. Unexpectedly, failure to cluster led to the generation of multipolar spindles that divided abnormally and generated aneuploid daughter cells. These cells died of apoptosis in a p53-dependent manner, depleting in this way the population of neural progenitors. These results suggest that multipolarity, aneuploidy and consequent cell death can also be at the basis of microcephaly. In agreement, mutations in *STIL*, identified in microcephaly patients, cause centrosome amplification (Arquint and Nigg 2014), and mutations in *Wdr62* cause spindle multipolarity (Chen et al. 2014). In addition, aneuploidy and cell death by apoptosis were also noticed in *asp* (*ASPM* orthologue) *Drosophila* mutants that present defects in head size (Rujano et al. 2013).

Lack of centrosomes or mutations perturbing its integrity frequently result in lengthened mitosis and increased mitotic index as the generation of a bipolar spindle in the absence of centrosomes takes more time (Basto et al. 2006; Bazzi and Anderson 2014; Chen et al. 2014; Insolera et al. 2014; Lizarraga et al. 2010; Sir et al. 2013). Although at the moment a true correlative relationship between mitotic delay and organ size has not been established, it is possible that certain phases of development would require fast proliferation rates at least in certain progenitor

cells. Failure to divide correctly or in a rapid way might induce cell death and also contribute to MCPH (Bazzi and Anderson 2014; Chen et al. 2014; Novorol et al. 2013) (see above).

Interestingly, cell cycle lengthening not related to centrosome dysfunction was proposed to lead to microcephaly. In Maier-Gorlin syndrome, for example, mutations in components of the pre-replicative complex cause defects in the initiation of DNA replication (Bicknell et al. 2011a; Bicknell et al. 2011b; Guernsey et al. 2011). As a consequence, S-phase progression and completion were delayed (Bicknell et al. 2011b). Recently, mutations in kinetochore components, *CASC5* (MCPH4) and *CENP-E* (MCPH13), have also been described in patients with severe microcephalic primordial dwarfism (Genin et al. 2012; Jamieson et al. 1999; Mirzaa et al. 2014). CASC5 is required for correct microtubule attachment to the centromere and the spindle assembly checkpoint (Kiyomitsu et al. 2007), whereas CENP-E is a kinesin required for accurate chromosome congression and segregation (Mirzaa et al. 2014; Putkey et al. 2002; Weaver et al. 2003). Therefore, aneuploidy appears as a possible cause of microcephaly in several size-related syndromes.

#### 5.4.1.2 Genes Identified So Far

Initially when the genetic causes of microcephaly started to be unravelled, it appeared that MCPH and SCKS could be classified not only phenotypically but also genetically (Table 5.1). *CPAP* and *CEP152* were first described as MCPH genes (Bond et al. 2005; Guernsey et al. 2010). Later, both were also identified in families affected by SCKS (Al-Dosari et al. 2010; Kalay et al. 2011). This led to the emerging idea that MCPH and SCKS are not two different diseases but a spectrum of the same disorder with different degrees of penetrance (Verloes et al. 1993). MOPD-II might also be included in this spectrum since *pericentrin* (*PCNT*) was initially reported in families with SCKS (Griffith et al. 2008) and subsequently in patients diagnosed with MOPD-II (Rauch et al. 2008; Willems et al. 2010). For this reason, we will refer to all these syndromes as primordial microcephalic disorders.

To date, twelve centrosome/spindle pole-related genes (Table 5.1) have been identified in primordial microcephalic disorders in humans. Mutations are frequently predicted to result in shorter truncated versions of the affected proteins. Most of our knowledge comes from studies performed in cell lines, and today, we still lack cell or animal models that recapitulate the human mutations as in most cases the few models available are knockdown or knockout approaches that decrease the overall level of wild-type proteins.

Here we describe the known functions of centrosome, spindle pole with mutation described in growth disorders. We will also briefly describe other genes associated with the microtubule cytoskeleton mutated in growth disorders.

**Table 5.1** Genes involved in diseases characterised by microcephaly

| Gene | Localisation | Disorder | References |
|---|---|---|---|
| MCPH1/ *microcephalin* | Chromatin associated | MCPH | Alderton et al. (2006), Jackson et al. (2002) Jackson et al. (1998) |
| MCPH2/ *WDR62* | Spindle pole component | MCPH cases with brain malformations | Bilguvar et al. (2010), Chen et al. (2014), Nicholas et al. (2010), Yu et al. (2010) |
| MCPH3/ *CDK5RAP2* (*Cep215*) | Centrosome (PCM component) | MCPH | Barrera et al. (2010), Bond et al. (2005), Buchman et al. (2010), Lancaster et al. (2013), Lizarraga et al. (2010) |
| MCPH4/ *CASC5* | Kinetochore | MCPH | Genin et al. (2012), Jamieson et al. (1999) |
| MCPH5/ *ASPM* | Spindle pole component | MCPH | Bond et al. (2002), Darvish et al. (2010), Pattison et al. (2000), Rujano et al. (2013) |
| MCPH6/ SCKL4/ *CPAP* | Centriole component | MCPH SCKS | Al-Dosari et al. (2010), Bazzi and Anderson (2014), Bond et al. (2005), Insolera et al. (2014), Kitagawa et al. (2011a) |
| MCPH7/*STIL* | Centriole component | MCPH | Arquint and Nigg (2014), Kumar et al. (2009), Novorol et al. (2013) |
| MCPH8/ *CEP135* | Centriole component | MCPH | Hussain et al. (2012) |
| MCPH9/ SCKL5/ *CEP152* | Centriole and PCM component | MCPH SCKS | Guernsey et al. (2010), Kalay et al. (2011) |
| MCPH10/ *ZNF335* | Chromatin remodelling protein | MCPH with MCD | Yang et al. (2012) |
| MCPH11/ *PHC1* | Chromatin remodelling protein | MCPH | Awad et al. (2013) |
| MCPH12/ *CDK6* | Cytoplasmic and nuclear (interphase), centrosome (mitosis) | MCPH | Hussain et al. (2013) |
| MCPH13/ *CENP-E* | Kinetochore | Similar to MOPD-II | Mirzaa et al. (2014) |
| SCKL6/ *CEP63* | Ring around parental centriole | SCKS | Sir et al. (2011) |
| *PLK4* | Centriole duplication regulator | SCKS with retinopathy | Martin et al. (2014), Shaheen et al. (2014) |
| *SAS-6* | Centriole component | MCPH | Khan et al. (2014) |
| *PCNT* | Centrosome (PCM component) | SCKS MOPD-II | Griffith et al. (2008), Rauch et al. (2008) |
| *LIS1* | MTs and spindle | LIS | Hattori et al. (1994), Moon et al. (2014), Reiner et al. (1993), Yingling et al. (2008) |
| *DCX* | MTs and spindle | LIS | des Portes et al. (1998), Gleeson et al. (1998) |

(continued)

**Table 5.1** (continued)

| Gene | Localisation | Disorder | References |
|---|---|---|---|
| *KIF5C* | MTs and spindle | Microcephaly with MCD | Poirier et al. (2013) |
| *KIF2A* | MTs and spindle | Microcephaly with MCD | Poirier et al. (2013) |
| *DYNC1H1* | MTs and spindle | MCD (usually normocephaly) | Poirier et al. (2013) |
| *TUBA1A* | MTs and spindle | LIS to MCD | Tischfield et al. (2011) |
| *TUBB2B* | MTs and spindle | PMG | Tischfield et al. (2011) |
| *TUBB3* | MTs and spindle | MCD | Tischfield et al. (2011) |
| *TUBG1* | MTs and spindle | Microcephaly with MCD | Poirier et al. (2013) |

The table shows all the genes described so far to be involved in diseases characterised by microcephaly. Genes called MCPH (MCPH 1–13) have been found mutated in patients affected by autosomal recessive primary microcephaly (MCPH). MCPH presents only minor brain malformations. In Seckel syndrome (SCKS) and microcephalic osteodysplastic primordial dwarfism type II (MOPD-II), microcephaly is accompanied with more severe defects such as polymicrogyria, retinopathy or defective neuronal migration. As described in the second column, most of the microcephalic genes encode for centrosomal proteins (centriole structure and PCM) or proteins associated with the mitotic spindle machinery

*MCPH* autosomal recessive primary microcephaly, *SCKS* Seckel syndrome, *MOPD-II* microcephalic osteodysplastic primordial dwarfism type II, *LIS* lissencephaly, *MCD* malformations of cortical development, *PMG* polymicrogyria

#### 5.4.1.3 Genes Required for Centriole Duplication

1. *CEP152* (known as asterless in flies) is associated with centrioles, and it is required for centriole duplication as it forms a scaffold for the recruitment of PLK4, the master regulator of centriole duplication (Cizmecioglu et al. 2010; Dzhindzhev et al. 2010; Hatch et al. 2010; Kim et al. 2013; Sonnen et al. 2013). In flies it is also required for PCM recruitment (Varmark et al. 2007). *CEP152* mutations were initially identified in patients affected by MCPH (Guernsey et al. 2010) and later also in families with SCKS (Kalay et al. 2011). Mutations are predicted to give loss-of-function truncated proteins. Analysis of fibroblasts and lymphocytes derived from SCKS patient cells showed increased replicative stress and chromosomal instability. In addition, high frequency of abnormal cell divisions with multiple nuclei, fragmented centrosomes and aneuploidy was also noticed (Kalay et al. 2011).
2. *PLK4* is a serine-threonine kinase, member of the polo-like kinase family. Its activity is required for centriole duplication (Bettencourt-Dias et al. 2005; Habedanck et al. 2005). PLK4 self-regulates its own stability through trans-autophosphorylation upon homodimerisation (Guderian et al. 2010; Holland et al. 2010). Two recent studies described mutations in *PLK4* in distinct families (Martin et al. 2014; Shaheen et al. 2014). Individuals displayed profound microcephaly, reduced stature and retinopathy. This latter defect was reported

for the first time in a primordial microcephalic gene (Martin et al. 2014). Patient-derived fibroblasts showed highly reduced PLK4 protein levels and impaired centriole duplication. Although spindle formation was affected, chromosome segregation defects were rarely observed. In a zebra fish model, depletion of *plk4* transcript through morpholino antisense oligonucleotides recapitulated the patients' phenotype: delay in mitotic progression, decreased cell number and consequently also body size reduction. Interestingly, cilia-related phenotypes were seen in a morpholino dose-dependent manner.

3. *CPAP* (known as SAS-4 in flies and worms) is required for microtubule attachment to the initial pro-centriole scaffold, and it controls centriole length and microtubule elongation (Pelletier et al. 2006; Tang et al. 2009). As *CEP152*, mutations in *CPAP* can also lead to both MCPH and SCKS syndromes (Al-Dosari et al. 2010; Bond et al. 2005). A mouse model expressing a truncated variant of CPAP recapitulates many clinical characteristics of SCKS, including intrauterine growth retardation, microcephaly and skeletal defects (McIntyre et al. 2012). DNA damage and apoptosis were also increased in the brain region where cortical neurogenesis takes place and the number of neurons was significantly reduced. A new mouse *Cpap* model, in which *Cpap* was selectively removed from neural progenitors during neurogenesis, also showed a strong microcephalic phenotype (Insolera et al. 2014). Loss of centrioles led to detachment of the neural progenitors from the ventricular zone, where they normally reside. Remarkably, these cells did not change their fate and maintained proliferative capacity. Nevertheless, mitosis was delayed and p53 expression was up-regulated. This led to apoptosis and consequently neuronal loss and microcephaly. Importantly, aneuploidy and DNA damage were not observed (Insolera et al. 2014). The expression of *CPAP* MCPH-mutated versions in human culture cells induced defects in centriole formation and randomised spindle orientation (Kitagawa et al. 2011a). Importantly, one CPAP mutation found in MCPH family impairs centriole formation *in vivo* (Kumar et al. 2009) due to a weaker interaction with STIL (Cottee et al. 2013).
4. Very recently, a mutation in the *HsSAS-6* gene that encodes a protein recruited during the initial steps of procentriole assembly (Kleylein-Sohn et al. 2007; Leidel et al. 2005; Strnad et al. 2007) has been reported in a newly identified MCPH family. This mutation, when expressed in human cells in culture, impaired centrosome duplication, which led to monopolar spindle formation (Khan et al. 2014). So it is possible that in this case abnormal cell division, aneuploidy and consequent cell death of neuronal progenitors contribute to brain size defects.
5. *STIL* (Ana2 and SAS-5 in *Drosophila* and *C. elegans*) is a centriole duplication protein that participates in cartwheel assembly (Arquint et al. 2012; Tang et al. 2011). STIL dissociation from centrosomes during early mitosis triggers HsSAS-6 dissociation and so cartwheel disassembly (Arquint and Nigg 2014). Mutations found in MCPH patients result in the expression of truncated proteins that lack the degradation motif and cause centrosome amplification (Arquint and Nigg 2014; Kumar et al. 2009). Likely, extra centrosomes and consequent

aneuploidy and cell death contribute to MCPH in this case. In addition, since centrosome amplification also causes a delay in mitosis (Basto et al. 2008; Marthiens et al. 2013), it is possible that this also contributes to brain size reduction as discussed above. Interestingly, morpholino-mediated knockdown in zebra fish showed a dramatic increase in both the number of retina progenitors and mitotic cells arrested in prometaphase and increase in apoptotic cells (Novorol et al. 2013).

6. *CEP135* (*Bld10* in flies) has been proposed to act as a bridging molecule between the "cartwheel" and centriole microtubules, being required for CPAP-mediated centriole elongation (Lin et al. 2013). A single mutation has been found so far in one MCPH family. This mutation results in a truncation at the C-terminus (Hussain et al. 2012), which affected the region that mediates CEP135-cartwheel interaction (Lin et al. 2013). Unexpectedly, 22 % of primary fibroblasts derived from patients completely lack centrosomes, while 18 % contained extra centrosomes, or centrosome fragments (Hussain et al. 2012). In this case, it is possible that several types of defects are at the basis of brain size reduction.
7. *CEP63* is an MCPH protein that regulates CEP152 centrosomal localisation to ensure efficient and timely controlled centriole duplication (Brown et al. 2013; Sir et al. 2011). Indeed, human B lymphocytes derived from affected patients showed reduced level of CEP152 at the centrosomes but without major defects in spindle formation and centrosome number (Sir et al. 2011). DT40 chicken B lymphocytes, which have a rapid cell cycle, presented an increased population doubling time and monopolar spindles due to inefficient centriole duplication. It has been proposed that CEP63 is required to timely ensure the presence of enough CEP152 (and consequently PLK4) to allow centriole duplication. Since neural progenitors divide much faster than lymphocytes (10–12 h vs. 24 h), the presence of CEP63 might be essential in the fast proliferating progenitors of the developing brain. Supporting this hypothesis, *Cep63*-deficient mice have neural progenitors with monopolar spindles and acentriolar spindle poles (Marjanovic et al. 2015). As a consequence, these defects delay mitosis, trigger p53-dependent cell death and ultimately lead to microcephaly, similar to the *Cpap*-mutant mouse model (Insolera et al. 2014). Moreover, *Cep63*-deficient mice also showed body growth retardation, recapitulating thus two key characteristics of human SCKS syndrome caused by *CEP63* mutations (Sir et al. 2011). Interestingly, this work also uncovered a surprising function of CEP63 in meiotic male recombination (Marjanovic et al. 2015). The authors proposed that centrosome loss in *Cep63*-deficient spermatocytes impairs normal intranuclear chromosome movement that is required to facilitate homologous chromosomes encounter and thus meiotic DNA recombination, leading to defective spermatogenesis. CEP63 seems to be also a target of the DNA damage response pathway in vertebrate cells. Activation of this pathway promotes CEP63 displacement from spindle poles, inhibiting spindle formation and delaying mitotic progression (Smith et al. 2009). Thus, mutations in CEP63 might perturb cell cycle progression in several ways.

#### 5.4.1.4 Genes Encoding for Centrosomal Proteins

8. *Pericentrin* (PCNT) is a component of the pericentriolar material (PCM), known to play an important role in the recruitment of proteins to the centrosome (e.g. γ-tubulin) (Doxsey et al. 1994; Zimmerman et al. 2004). It was the first centrosomal gene identified in two primordial dwarfism disorders, SCKS and MOPDII (Griffith et al. 2008; Rauch et al. 2008). In lymphoblastoid SCKS patient cell lines defective ATR signalling pathway and lack of G2-M checkpoint after UV radiation were also reported (Griffith et al. 2008). Importantly however, monopolar spindles with decreased γ-tubulin recruitment were also noticed, raising the possibility that these abnormal spindles also contribute to abnormal chromosome segregation and aneuploidy in cells with PCNT mutations.
9. *CDK5RAP2* (Cep215 and Cnn) is a PCM protein involved in γ-tubulin recruitment (Fong et al. 2008). CDK5RAP2 seems to be involved in centriole engagement and maintenance of the neural progenitor pool in the mouse developing neocortex (Barrera et al. 2010; Buchman et al. 2010). Embryonic fibroblasts derived from mouse models carrying *Cdk5rap2* mutations similar to the ones found in human MCPH showed centrosome amplification due to loss of centriole engagement and consequent formation of multipolar spindles (Barrera et al. 2010). *Cdk5rap2* knockdown by *in utero* electroporation described a depletion of neural progenitors in the developing mouse neocortex due to premature neural differentiation (Buchman et al. 2010). However, in these two studies, neither spindle orientation nor microcephaly was observed. Importantly, an *in vitro* model of human brain development that used reprogrammed skin fibroblasts from MCPH patients showed reduced neuroepithelial tissue with defects in spindle orientation and premature neural differentiation (Lancaster et al. 2013). Spindle positioning might, however, not be the sole defect as the characterisation of Hertwig's anaemia mouse model, which carries a mutation in the *Cdk5rap2* gene, also showed multipolar spindles in neural progenitors accompanied by cell death (Lizarraga et al. 2010).
10. *CDK6*, in concert with CDK4, regulates the G1/S transition (Meyerson and Harlow 1994). It localises in the cytoplasm and in the nucleus in interphase and also at the centrosome throughout mitosis (Hussain et al. 2013). Fibroblasts from MCPH patients do not contain centrosomal CDK6 during mitosis. This results in several defects such as disorganised interphase microtubule network and mitotic spindles, centrosome amplification, reduced proliferation and cell death (Hussain et al. 2013). Although *Cdk6*-null mice do not show microcephaly at birth, CDK6 is required during adult neurogenesis. Lack of this kinase resulted in lengthened G1 and consequent premature cell cycle exit (Beukelaers et al. 2011; Malumbres et al. 2004). Absence of microcephaly in *Cdk6*-null mice suggests that the particular mutation found in MCPH patients might have a more severe effect in brain development than loss of CDK6.

#### 5.4.1.5 Genes Encoding for Spindle Pole-Associated Proteins

11. The abnormal spindle-like microcephaly-associated (*ASPM*) gene is the most frequently mutated locus found in MCPH (Bond et al. 2002; Darvish et al. 2010; Pattison et al. 2000). ASPM is a microtubule minus end- and spindle pole-associated protein with important roles in cell division (do Carmo Avides and Glover 1999; Gonzalez et al. 1988; Gonzalez et al. 1990; Riparbelli et al. 2002; Saunders et al. 1997; Wakefield et al. 2001). Loss of ASPM causes alteration in spindle positioning in mouse neural stem cells, which favours asymmetric cell division depleting the pool of progenitors (Fish et al. 2006). Recent work in *Drosophila* showed that the ASPM orthologue, Asp, also plays a role in brain size regulation in flies (Rujano et al. 2013). Defects in spindle orientation, chromosome segregation and interkinetic nuclear migration were noticed. Moreover, Asp was found to interact with myosin II, and this interaction was essential during brain morphogenesis to maintain neuroepithelial organisation (Rujano et al. 2013). These results showed that Asp plays unexpected functions, beyond the role in microtubule cytoskeleton in brain development. They might also explain the observations that some MCPH mutations also affect brain organisation in addition to size (Mochida 2005).
12. *WDR62* is the second most common mutated gene in MCPH. It is a spindle pole protein-coding gene (Bilguvar et al. 2010; Nicholas et al. 2010; Yu et al. 2010). It has been recently demonstrated in a hypomorphic *Wdr62* mouse model that neural progenitor cells are arrested in mitosis due to spindle stability defects with increased cell death. In addition, defects in spindle positioning or premature differentiation were not seen, suggesting that disruption of mitotic progression and consequent cell death of neural progenitors is a potential cause of human microcephaly (Chen et al. 2014).

#### 5.4.1.6 Genes Encoding for Molecular Motors and Microtubule-Associated Proteins

Defects in molecular motors and microtubule-associated proteins lead to severe disorders with microcephaly and brain malformation. Lissencephaly is characterised by the absence of normal folds in the cerebral cortex due to defective neuronal migration (Dobyns et al. 1993). The first gene identified in lissencephaly was *LIS1* (Hattori et al. 1994; Reiner et al. 1993), which encodes a subunit of the cytoplasmic dynein complex. An *in vivo* study demonstrated its requirement for neuronal migration (Reiner et al. 1995). Interestingly, LIS1 has also been implicated in spindle positioning of apical neural progenitors in mouse (Yingling et al. 2008), and recently centrosome amplification and severe chromosome segregation defects have also been described in *Lis1* mutant MEFs (Moon et al. 2014), suggesting that aneuploidy and cell death might also contribute to the overall phenotype. Neuronal migration is impaired in mutation in *Doublecortin* (*DCX*),

and patients carrying this mutation also present lissencephaly (des Portes et al. 1998; Gleeson et al. 1998).

Mutations in *KIF5C* and *KIF2A*, members of the kinesin superfamily, and in *DYNC1H1*, cytoplasmic dynein 1 heavy chain 1, have also been recently described in patients affected by lissencephaly with microcephaly (Poirier et al. 2013). These mutations affect ATP hydrolysis, protein folding and microtubule binding.

Mutation in another motor, *KIF11* (kinesin Eg5), was found to lead to syndromes characterised by microcephaly accompanied by eye malformations (Ostergaard et al. 2012). Eg5 is a mitotic kinesin involved in centrosome separation (Kwok et al. 2004) and centrosome clustering (Drosopoulos et al. 2014). These results suggest that neural progenitors are more vulnerable to microtubule mutations than other cell types during embryonic development. A further demonstration of the importance of functional microtubules was given by the fact that mutations in *α*-, *β*- and *γ-tubulin* isotypes coding genes also lead to microcephaly with brain malformations (Poirier et al. 2013; Tischfield et al. 2011). Importantly, all human mutations identified in these genes are heterozygous missense mutations.

For further discussion of neurodevelopmental defects caused by an impaired microtubule cytoskeleton, please also see the Chap. 4 by Sánchez-Huertas, Freixo and Lüders.

#### 5.4.1.7 Genes Encoding for Chromatin Associated Proteins

*Microcephalin* (MCPH1) was the first mutated locus identified in patients affected by MCPH (Jackson et al. 1998). Microcephalin is highly expressed in the developing mouse forebrain, in particular in the region where neural progenitors reside (Jackson et al. 2002). It localises to the DNA during interphase, and it has a role in chromosome condensation. Furthermore, microcephalin mediates the DNA damage response, being recruited to the damaged foci (Lin et al. 2005; Rai et al. 2006; Xu et al. 2004). MCPH1 also localises at centrosomes in U2OS cells (Zhong et al. 2006), in chicken DT-40 cells after irradiation (Jeffers et al. 2008), and recruits Chk1, a kinase involved in the G2-M checkpoint (Alderton et al. 2006). Importantly, however, human lymphoblastoid cell lines with truncating mutations found in MCPH patients do not show impaired DNA damage response, but rather a defective G2-M checkpoint. In these cells, Chk1 is not targeted to the centrosome and mitosis starts even in the presence of damaged DNA, leading to nuclear fragmentation and centrosome amplification (Alderton et al. 2006). Studies from *Drosophila* suggested a role for MCPH1 (also known as *awol*) in chromosome condensation but not in the DNA damage response (Brunk et al. 2007; Rickmyre et al. 2007). A centrosomal localisation of MCPH1 during mitosis has been reported in *Drosophila* embryos (Brunk et al. 2007). However, different to all the other centrosomal/spindle pole MCPH genes, a clear spindle function has not been identified.

In addition to cytoskeleton genes, mutations in the nuclear zinc finger 335 (*ZNF335*, trithorax group) and *PHC1* (polycomb group) genes have been

recently identified in families with severe microcephaly, suggesting a broader cause resulting from alterations in gene expression (Awad et al. 2013; Yang et al. 2012).

### 5.4.2 Centrosome Defects and Cancer

Centrosome defects and in particular centrosome amplification are usually linked to cancer. Centrosome amplification is present in almost all solid and haematological tumours described (Chan 2011) (Fig. 5.4). At the beginning of the twentieth century, German zoologist Theodor Boveri proposed that centrosome amplification and consequent aneuploidy could be at the basis of tumour initiation (Boveri 2008). This hypothesis was proposed after the observations that the presence of extra centrosomes in sea urchin embryos, due to dispermic fertilisation, could lead to abnormal mitosis and defects in chromosome segregation. These defective chromosome combinations were usually detrimental for embryo development, but Boveri could observe rare cases where abnormal cells continued to proliferate. Boveri remarkably found that they were similar to tumour cells (Boveri 2008). At his time it was already known that aneuploidy was a characteristic of human tumours. Indeed, in 1890 David Hansemann initially observed asymmetric chromosome segregation in human epithelial cells, and he documented this phenomenon in a variety of tumours (Boveri 2008).

Persistent high levels of chromosome mis-segregation, commonly referred to as "chromosomal instability" (CIN), are hallmark of most cancers (Lengauer et al. 1997). It is difficult to understand how centrosome amplification contributes to CIN, since multipolarity is often associated with poor viability. A link between centrosome amplification and viable CIN has been established recently. The transition from multipolarity to bipolarity during the process of clustering promotes merotelic attachments that might lead to viable aneuploid daughter cells (Ganem

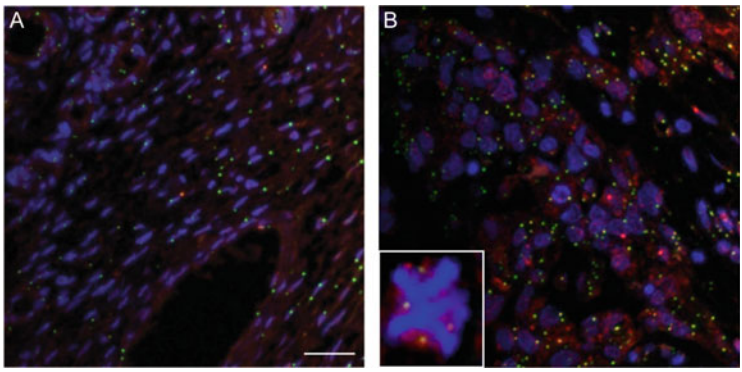


**Fig. 5.4** Centrosome amplification in human ovarian tumour. Human ovarian tissue (**a**) and serous tumour (**b**) sections immunostained for pericentrin (*green*), γ-tubulin (*red*). DNA is shown in *blue*. Scale bar = 30 μm

et al. 2009; Silkworth et al. 2009). The contribution of aneuploidy (generated independently of centrosome amplification) to tumorigenesis is tissue dependent. While aneuploidy can promote tumour formation in certain tissues upon carcinogenic treatment, it can also inhibit tumorigenesis in other tissues (Silk et al. 2013; Sotillo et al. 2007; Weaver et al. 2007).

Centrosome amplification was shown to initiate tumorigenesis in *Drosophila* both independently and dependently of aneuploidy. Allograft transplantation of larval brains carrying extra centrosomes caused over-proliferation and tumours. Although defects in chromosome segregation were not observed, defects in mitotic spindle positioning resulted in the increase of the neural stem cell pool (Basto et al. 2008). In flies, mutations that perturb neural stem cell asymmetric cell division due to centriole duplication defects or mutations in polarity genes are tumorigenic with little if any CIN (Castellanos et al. 2008; Caussinus and Gonzalez 2005). In another tissue, the wing imaginal disc, extra-centrosomes, are not efficiently clustered or inactivated with consequent multipolar spindle formation. This generates aneuploid cells and causes tumours in allograft transplantation (Sabino et al. 2015).

In vertebrates the contribution of centrosome amplification to tumorigenesis is still an open question. Overexpression of *Plk4*, which still remains the most efficient mean to drive centriole over-duplication *in vivo*, in the mouse developing central nervous system resulted in microcephaly (Fig. 5.2), but brain tumours were not reported (Marthiens et al. 2013). Although centrosome clustering allows the assembly of bipolar spindles in most embryonic neural stem cells, in a significant proportion of cells, mainly during early and mid-neurogenesis, tripolar spindles and abnormal chromosome segregation lead to the generation of unviable aneuploid cells. These cells died by apoptosis in a p53-dependent manner. Importantly, even in the absence of p53, tumours were not detected in the CNS. It is therefore possible that during development, centrosome amplification and aneuploidy are not sufficient to initiate tumour formation. It will be important in the future to establish whether centrosome amplification during adult life in the mammalian brain or in highly proliferative tissues such as the intestine or the skin is able to drive tumour formation.

Until recently, centrosome amplification was thought to only contribute to tumour formation through the generation of aneuploidy and spindle positioning defects. However, it is also possible that the presence of extra centrosomes even in interphase cells might represent an advantageous condition. Surprisingly, non-transformed human mammary epithelial cells with extra centrosomes showed increased microtubule nucleation capacity that strongly correlated with invasive behaviour (Godinho et al. 2014). Increased centrosomal microtubule nucleation during interphase activates the small GTPase Rac1, which is known to promote invasiveness and metastasis (Mack et al. 2011). It is therefore possible that centrosome amplification contributes to tumour formation in several different ways.

## 5.5 Conclusions

At the beginning of the last century, it was proposed that defects in centrosome number, in particular centrosome amplification, might be a cause for tumour formation (Boveri 2008). Today, several lines of evidence support this view (Basto et al. 2008; Castellanos et al. 2008; Ganem et al. 2009; Godinho et al. 2014; Nigg 2006; Sabino et al. 2015; Zyss and Gergely 2009). However, the observations made during the past 15 years using autozygosity mapping techniques and whole-genome SNP genotyping implicated centrosome mutations in growth defective syndromes, but not in cancer.

Both primordial dwarfisms and MCPH are characterised by proportionate reduction of body or head size, which results from premature depletion of progenitors and/or increased levels of cell death. In most tumours, cancer cells show high levels of proliferation, and even if high cell death rates can be identified, proliferation and capacity to evade cell death signals are essential during cancer progression and invasion (Hanahan and Weinberg 2000; Hanahan and Weinberg 2011). It is important to mention that most of the cellular pathways affected in MCPH or in primordial dwarfisms are also frequently referred to be dysfunctional in cancer cells. This is the case for DNA damage response (Lord and Ashworth 2012), spindle orientation (Gonzalez 2007) or abnormal cell division and aneuploidy (Boveri 2008). It is therefore possible that these conditions just represent two sides of the same coin. Centrosome dysfunction due to zygotic mutations, if viable, would lead to growth defects such as MCPH or dwarfism. If acquired in somatic adult tissues in certain contexts, they might lead to the opposite effect: over-proliferation and growth. Interestingly, mutation in *BUBR1* and *CEP57* (kinetochore and centrosomal proteins, respectively) leads to a disease called mosaic variegated aneuploidy (MVA), which is characterised by the appearance of tumours at early age and features of primordial dwarfism (microcephaly and short stature) (Hanks et al. 2006; Snape et al. 2011).

Further work is required to understand the relation between centrosome mutations with cancer, MCPH and primordial dwarfism. Can centrosome amplification or any other types of centrosome dysfunction initiate tumorigenesis in humans? And if yes, by which means? Which adult tissues are more prone to develop cancer when accumulating centrosome defects? And concerning growth defects, why is the brain the most susceptible tissue to centrosome mutations? For all these reasons, the centrosome field remains an active one and calls for *in vivo* investigations that will keep us busy in the years to come.

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