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Review

Review

The DNA damage response molecule MCPH1 in brain development and beyond

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Abstract

Microcephalin (MCPH1) is identified as being responsible for the neurodevelopmental disorder primary microcephaly type 1, which is characterized by a smaller-than-normal brain size and mental retardation. MCPH1 has originally been identified as an important regulator of telomere integrity and of cell cycle control. Genetic and cellular studies show that MCPH1 controls neurogenesis by coordinating the cell cycle and the centrosome cycle and thereby regulating the division mode of neuroprogenitors to prevent the exhaustion of the progenitor pool and thereby microcephaly. In addition to its role in neurogenesis, MCPH1 plays a role in gonad development. MCPH1 also functions as a tumor suppressor in several human cancers as well as in mouse models. Here, we review the role of MCPH1 in DNA damage response, cell cycle control, chromosome condensation and chromatin remodeling. We also summarize the studies on the biological functions of MCPH1 in brain size determination and in pathologies, including infertility and cancer.

Key words: MCPH1, DNA damage response, cell cycle, microcephaly, neurogenesis

Brain Evolution and Primary Microcephaly

The volume of the human brain has greatly enlarged during the evolutionary path from early primates to hominids and finally to *Homo sapiens*. This increase has been seen along the human timeline of evolution, starting from ~600 cm^3 in *Homo habilis* up to 1500 cm^3 in *Homo sapiens neanderthalensis*, which is the hominid with the biggest brain size [1,2]. Brain development is a dynamic process that requires a complex molecular network to coordinate the genesis of specific cell types, the maintenance and homeostasis of stem cells, and the wiring of different cells within the brain structure. Many molecular pathways, such as gene transcription, cell cycle control, epigenetic modulation, and DNA damage response (DDR), have been intensively studied in the brain development. Given its function in controlling cell proliferation, cell cycle progression, and cell death, a proficient DDR is vital in brain development and also in age-related neuropathy, including neurodegeneration. An aberrant response to DNA lesions is implicated in many human neurodevelopmental and also neurodegenerative disorders [3–5].

Microcephaly, a human autosomal recessive disorder, is characterized clinically by a reduction of head circumference of more than 3 SD compared to normal individuals [6–8]. Microcephaly is divided into two categories: primary microcephaly, which is a prenatal developmental neurogenic disorder, and secondary microcephaly, which is associated with progressive neurodegenerative diseases.

Human primary microcephaly (MCPH, OMIM251200) is a neurodevelopmental disorder characterized by a marked reduction in brain size, especially by a reduced cerebral cortex, but with a normal architecture and non-progressive mental retardation with an IQ between 30% and 80% of the average [9]. The incidence of MCPH is 1:30,000–1:250,000 per live-birth depending on the population [7]. MCPH is rarer in Whites than in Asian and Arab populations where consanguineous marriages are common [10]. So far, 12 gene loci have been identified to be responsible for MCPH, including *MCPH1/BRIT1* (microcephalin 1/BRCT-repeats inhibitor of hTERT expression), *WDR62* (WD-40 repeat protein 62), *CDK5RAP2* (cyclin-dependent kinase 5 regulatory associated protein 2), *CASC5* (cancer susceptibility candidate 5), *ASPM* (abnormal spindle-like microcephaly associated ASPM), *CENPJ* (centromeric protein J), *STIL* (SCL/TAL1-interrupting

locus protein), *CEP135* (centrosome protein 135 kD), *CEP152* (centrosome protein 152 kD), *ZNF335* (zinc finger protein 335), *PHC1* (polyhomeoric-like 1 protein), and *CDK6* (cyclin-dependent kinase 6), which have been discussed in previous review articles [11,12].

Discovery of the *MCPH1* Gene

The *MCPH1* gene, located in the human chromosome 8p23.1, exhibits a positive selection during the evolution of human and non-human primates [13,14]. *MCPH1* is the first gene reported to cause primary microcephaly in two consanguineous Pakistani families and was later identified to be a causal gene for another syndrome called premature chromosome condensation syndrome (PCC, OMIM 606858) [6,15–17]. Indeed, MCPH1 patient cells exhibit a prophase-like and premature chromosome condensation [18,19]. The *MCPH1* sequence later is found to be identical to BRIT1, a gene identified in an independent genetic screen for the transcriptional repressors of hTERT (the catalytic subunit of human telomerase) [20].

MCPH1 mRNA and gene expression

The human *MCPH1* gene contains 14 coding exons. In addition to the full length of the MCPH1 (MCPH1-FL) transcript, three alternative transcripts were identified in the human 562 T fibroblasts: MCPH1Δe1–3, Δe9–14, and Δe8 [6,21] (**Fig. 1**). MCPH1Δe1–3 lacks the first three exons due to the usage of an alternative transcriptional start site in exon 4, which results in the loss of the N-terminal BRCA1 C-terminus (BRCT) domain. However, MCPH1Δe1–3 may not be physiologically expressed [20]. MCPH1Δe9–14 derives from a premature polyadenylation signal within intron 8, creating a new stop codon downstream of the original exon 8, which deletes the two C-terminal BRCT domains with a predicted molecular weight of around 70 kDa. MCPH1Δe8 is a novel isoform recently discovered in human cells [21]. It lacks exon 8 due to a weak 5′-splice site in intron 8, resulting in a protein containing all three BRCT domains, but not the canonical nuclear localization signal (NLS) motif (**Fig. 1**). This isoform contains 450 amino acids with a predicted molecular weight of ~50 kDa [6,21].

MCPH1 is ubiquitously expressed in adult human tissues including the brain, testes, pancreas, and liver. The three MCPH1 variants are differentially expressed in human fetal and adult tissues [6,22]. *In situ* hybridization reveals that *Mcph1* is highly expressed in the fetal forebrain of the mouse, but declines after birth [23]. Both MCPH1-FL and MCPH1Δe9–14 are highly expressed in the fetal stage (e.g. in the brain, heart, and thymus) compared to the respective adult samples [21]. However, MCPH1Δe8 is expressed at a lower level compared to MCPH1-FL and MCPH1Δe9–14 in all organs [21]. Moreover, these three variants are differentially expressed during cell cycle progression. While MCPH1-FL mRNA decreases from the mid S to the G2 phase, MCPH1Δe9–14 is reduced during the early S phase but increased during the late S and G2 phase. The MCPH1Δe8 transcript is detected at low levels at all cell cycle phases [21].

MCPH1 protein structure and function

The human *MCPH1* gene encodes an MCPH1-FL of the 835 aa (amino acid) with ~110 kDa molecular weight. MCPH1-FL contains three BRCT domains, one in the N-terminus that extends from 1 aa to 93 aa, and the other two in the C-terminus, from 672 aa to 730 aa and from 751 aa to 833 aa, respectively [6,22,24] (**Fig. 1**). The BRCT domain is an evolutionarily conserved phosphopeptide-interacting region and frequently found in proteins involved in DDR signaling and cell cycle control. The three BRCT domains of MCPH1 have been shown to function in DDR. The N-terminal BRCT domain interacts with the chromatin remodeling complex SWItch/sucrose non-fermentable (SWI/SNF) in DNA repair [25] and is required for the centrosome localization of chicken MCPH1 in irradiated DT40 cells [24]. The tandem C-terminal BRCT domains are necessary for forming oligomer and ironizing radiation-induced foci by interacting with γH2AX [24].

The central region of MCPH1 is called central microcephalin protein domain, or IMPDH domain (inosine monophosphate dehydrogenase domain), for which a clear function has not been established to date. However, a condensin II-binding motif from the residue 376 to 485 in the IMPDH domain appears to be required for homologous recombination (HR) repair [26,27]. Evolutionary genetics studies demonstrate that an amino acid change in the IMPDH domain is associated with the brain enlargement during primate and human evolution [2,14], suggesting the central domain also plays an important role in brain development.

Subcellular localization of MCPH1

An immunostaining assay using the anti-MCPH1 antibody showed that the MCPH1 signal overlaps with the centrosome marker γ-tubulin [28]. Consistent with this, the green fluorescent protein (GFP)-tagged chicken MCPH1 localizes to the centrosome in chicken DT40 cells [24]. Additional evidence supporting this notion is the observation that MCPH1 deficiency results in centrosome amplification, chromosome misalignment, and lagging chromosomes with a deficient spindle checkpoint and cytokinesis in mammalian cells (U2OS cells, or human MCPH1 patient cells) and in chicken DT40 cells [29–31]. *Mcph1*-del neuroprogenitors also show multiple spindle poles, chromosome misalignment, and lagging chromosomes [23]. However, other studies failed to observe the centrosomal localization of MCPH1 in HeLa, 562 T, or HEK293T cells using tagged human MCPH1 [27,29,32]. Moreover, all three of the GFP-tagged MCPH1 isoforms are predominantly located in the nucleus but with a low level of cytoplasmic fraction [21].

Mutations in the human *MCPH1* gene

So far, a total of 15 different homologous mutations in the *MCPH1* gene have been identified as a cause of primary microcephaly. Interestingly, all mutations identified so far are located in the exons 1–6 (**Table 1**). Of note, all missense mutations of *MCPH1* are located in exons 2 and 3 that encode the N-terminal BRCT domain (**Table 1**), suggesting an important function of the N-terminal BRCT domain in brain development.

However, the two insertion mutations (c.427_428insA and c.566_567insA) [18,33] produce a truncated protein with an intact N-terminal BRCT domain but lacking the whole middle region and the C-terminal BRCT domain. In addition, a splice-acceptor site mutation (c322–2A > T) was recently identified in two children from a consanguineous Iranian family, which results in an RNA processing defect with a 15 nucleotide deletion in exon 5 (r.322–336del15, p.R108-Q112del5) [34]. This truncated protein contains both intact N-terminal and C-terminal BRCT domains but five amino acids are deleted in the middle region of MCPH1. These data indicate that the middle domain of MCPH1 is also important for brain development.

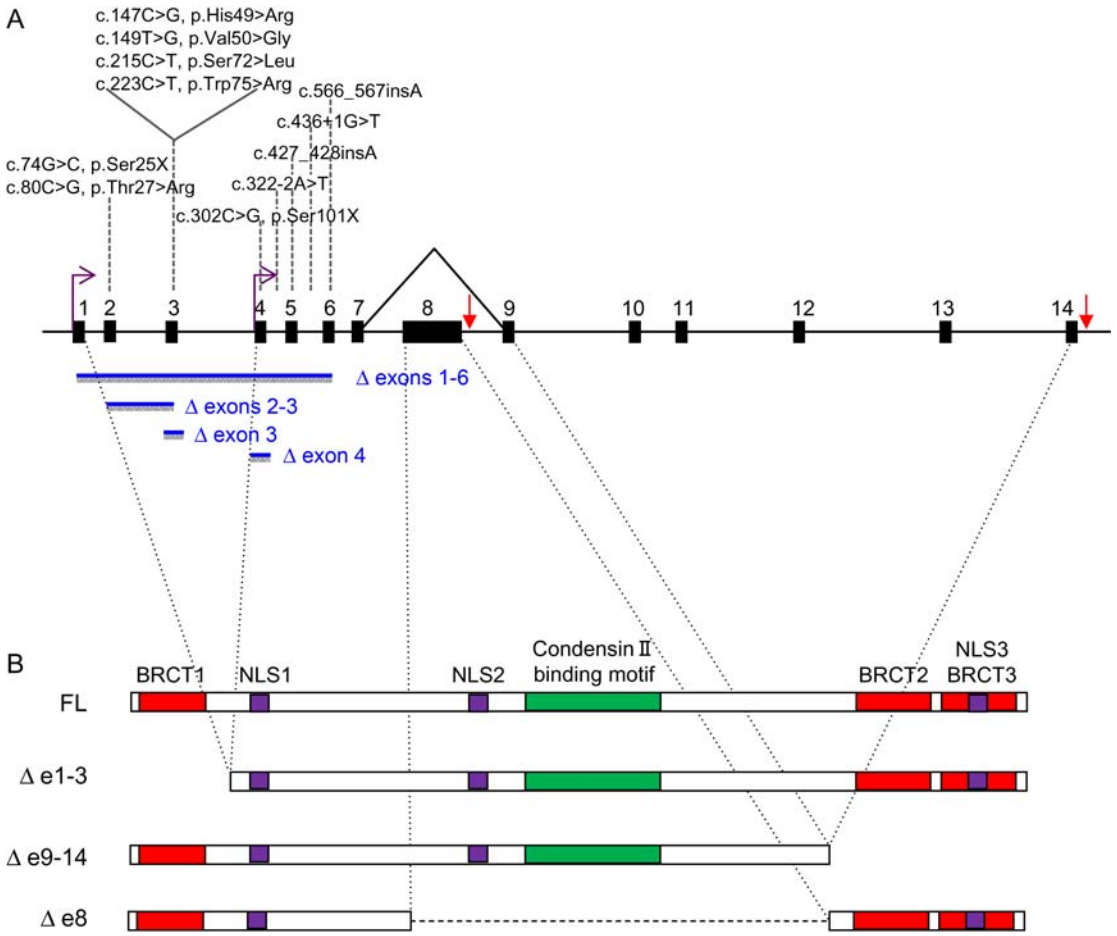

Figure 1. Schematic view of the MCPH1 gene isoforms and protein structure (A) Fourteen exons of the *MCPH1* gene are shown in the black box. Two transcriptional start sites located at exons 1 and 4 are shown by black curved rows. Red arrows indicate the positions of the regular (after exon 14) and of the alternative (after exon 8) polyadenylation sites poly(A), respectively. An alternative isoform from exons 7 to 9, skipping exon 8, is produced due to a weak 5′-splice site in intron 8. The reported point mutations in the *MCPH1* gene in human patients are depicted on the top of the gene. The deletion mutations are shown as blue bars below the gene. (B) Variants of MCPH1-FL, MCPH1Δe1–3, Δe9–14, and Δe8 are shown. Red boxes represent the BRCT domains, purple boxes represent NLS, and the green boxes indicate the condensin II-binding motif.

Table 1. MCPH1 mutations in human primary microcephaly patients

Type of mutations	Mutation sites	Location	Predicted protein effect
Nonsense mutation	c.74 G > C, p.Ser25X	Exon 2	Truncation
Nonsense mutation	c.302 C > G, p. Ser101X	Exon 4	Truncation
Insertion of one base	c.427_428insA	Exon 5	Truncation
Insertion of one base	c.566_567insA	Exon 6	Truncation
Splic site mutation	c.322–2 A > T	Intron 4	Deletion of five aa in exon 5
Splic site mutation	c.436+1 G > T	Intron 5	Truncation
Gross deletion	Deletion exons 2–3	Exons 2–3	Truncation
Gross deletion	Deletion exon 3	Exon 3	Truncation
Gross deletion	Deletion exon 4	Exon 4	Truncation
Gross deletion	Deletion the first six exons	Exons 1–6	Truncation
Missense mutation	c.80 C > G, p.Thr27 > Arg	Exon 2	Aa substitution
Missense mutation	c.147 C > G, p.His49 > Gln	Exon 3	Aa substitution
Missense mutation	c.149 T > G, p.Val50 > Gly	Exon 3	Aa substitution
Missense mutation	c.215 C > T, p.Ser72 > Leu	Exon 3	Aa substitution
Missense mutation	c.223 T > C, p.Trp75 > Agr	Exon 3	Aa substitution

Functions of MCPH1 in DDR and DNA Repair

MCPH1 has been shown to be a mediator for both ataxia telangiectasia mutated (ATM) and ATM-Rad3-related (ATR) pathways in response to DNA damage. Upon ionizing radiation (IR) or UV treatment, MCPH1 forms discrete nuclear foci and co-localizes with numerous proteins, including γH2AX, mediator of DNA-damage checkpoint 1 (MDC1), p53-binding protein 1 (53BP1), p-ATM, ATR, p-RAD17, and replication protein A (p-RPA34) [35,36]. A depletion of MCPH1 abolishes the IR-induced focus formation of MDC1, 53BP1, and p-ATM. The UV-induced phosphorylation of RAD17 and RPA34 is reduced in MCPH1-deficient cells, indicating a role of MCPH1 in the ATR pathway [35,36]. MCPH1 interacts with and recruits TopBP1 (topoisomerase-binding protein 1), an activator of ATR, in order to amplify the ATR signal under replication stress. The phosphorylation of MCPH1 at S322 by ATM and ATR is required for the recruitment of TopBP1 [37]. Moreover, the interaction between MCPH1 and the chromatin remodeling complex SWI/SNF is increased upon DNA damage in an ATR-dependent manner, which relaxes the chromatin structure and facilitates the access of repair proteins to the DNA damage sites [25]. MCPH1 can directly interact with BRCA2-Rad51 to execute HR repair [27,38]. In addition, MCPH1 interacts with the transcription factor E2F1 on the promoters of checkpoint kinase 1 (*CHK1*) and *BRCA1* to regulate their expression, indicating that MCPH1 can also function as transcription factor to participate in DNA repair [39]. Taken together, all these studies demonstrate that MCPH1 is a pleiotropic factor involved in DNA repair, genomic stability, and chromatin remodeling.

Functions of MCPH1 in Cell Cycle Control

A proliferating cell needs DNA to duplicate and to ensure an equal distribution of identical genetic information into two daughter cells. To control the quality of genomic integrity, cells evolve a mechanism called cell cycle checkpoint to examine the cellular machinery in response to DNA damage to prevent the propagation of damaged or wrong genetic information in their daughter cells. MCPH1 is implicated in the G2-M checkpoint by regulating the expression of CHK1 and BRCA1 [39,40]. A knockdown of MCPH1 by siRNA reduces the expression of CHK1 and BRCA1 [40]. Moreover, the level of CHK1 at the centrosomes is reduced in MCPH1-deficient lymphoblastoid cells leading to a premature entry into mitosis, because of the loss of a direct interaction of MCPH1 with PCNT (pericentrin) [41]. In fact, human MCPH1 patient cells with truncation mutations of MCPH1 also exhibit a defective G2-M checkpoint-mediated cell cycle arrest after DNA damage, which is characterized by an impaired degradation of cell division cycle 25A (Cdc25A) in an ATR-dependent manner [30]. MCPH1-deleted neuroprogenitors show a compromised CHK1 loading to the centrosome, which is associated with a premature activation of cyclin-dependent kinase 1 (Cdk1) and an early mitotic entry [23]. However, the expression of CHK1 or BRCT1 and the early ATR-dependent phosphorylation of these substrates are not affected in human MCPH1 patient cells [30,42]. Moreover, MCPH1 mutant cells, but not ATR-Seckel syndrome cells, show hypo-phosphorylated Cdk1, which drives the G2-M transition [30]. These data indicate that MCPH1, via ATR-CHK1-dependent and -independent pathways, maintains the inhibitory Cdk1 phosphorylation status to prevent premature mitotic entry.

MCPH1 and Brain Development: Lessons from Mouse Models

The development of the cerebral cortex is a well-organized process involving the formation and patterning of the neural tube, followed by neuroprogenitor cell proliferation and differentiation, neuron migration, and maturation. The brain size is primarily dependent on the capacity of neuroprogenitor proliferation and self-renewal [43,44].

To prove the causal role of MCPH1 in the microcephaly phenotype, genetic mouse models are perhaps the most suitable, albeit not the best, animal model. The mouse ortholog of MCPH1, containing 822 aa, has 57% homology to the human protein [6]. Several *Mcph1*-knockout mouse models have been generated [44]. The first *Mcph1*$^{-/-}$ mouse model was generated by deleting exon 2 of *Mcph1* in mice using the Cre/LoxP system. These mice show no obvious microcephaly but exhibit growth retardation with a body weight of only 80% compared to wild-type littermates [36]. Another prominent phenotype of these mice is sterility [36]. Another mouse model is called *Mcph1*$^{tg/tg}$, which was generated by a gene trap approach to insert a LacZ vector into intron 12, leading to a deletion of the C-terminal BRCT domains [45]. *Mcph1*$^{tg/tg}$ mice show dysregulated chromosome condensation and a reduced survival rate. However, the body weight and brain size of *Mcph1*$^{tg/tg}$ mice are within the range of the wild-type controls. No other pathologies were reported. Moreover, the DDR, such as the damage-induced foci of 53BP1 and H2AX, chromosomal breakage, and the G2-M checkpoint are all normal [45].

We generated null-*Mcph1* mice (*Mcph1*-del) by deleting exons 4 and 5 using the Cre/LoxP technology. *Mcph1*-del mice are viable but have a lower brain weight at birth compared to control littermates. The thinner neocortex in *Mcph1*-del mice is visible already at E13.5 [23]. The microcephaly phenotype is also reported by another mouse model referred to as *Mcph1*^{tm1a}, which was produced by deleting exon 4 of the *Mcph1* gene. *Mcph1*^{tm1a} mice show no evidence of retarded growth but have less brain weight. Another phenotype of *Mcph1*^{tm1a} mice is a mild to moderate hearing impairment with around 70% penetrance, suggesting a neurological defect [46].

The cell fate of neuroprogenitors is determined by symmetric and asymmetric division. A symmetric division of neuroprogenitors results in the generation of two identical neuroprogenitors or neurons, while an asymmetric division leads to the production of one progenitor and one neuron [34,43]. The homeostasis of symmetric and asymmetric division plays an important role in brain development and is regulated by many factors. Cell cycle length can influence the decision on symmetric or asymmetric division [47]. The transition of the progenitors from proliferative to neurogenic division during embryonic development is associated with an increase in the length of their cell cycle, especially in the G1 phase. Consequently, a relatively long G1 phase would switch from proliferation to neurogenic production, while a short G1 phase would promote cell cycle progression favoring self-renewal [47]. The spindle orientation or division cleavage plane is another important mechanism to regulate the symmetric and asymmetric division mode [48]. When the division cleavage plane is vertical to the apical surface of the neocortex, the neuroprogenitors undergo a symmetric division, leading to neuroprogenitor self-renewal; whereas when the cleavage plane is horizontal to the apical surface, the cells undergo an asymmetric division, which leads to neuroprogenitor differentiation [48].

The centrosome activity is important for the spindle alignment, which is an important regulatory mechanism for neuronal progenitor cell fate determination and brain development. So far, 10 out of the 12 gene loci have been identified to be responsible for MCPH, encoding proteins that associate with the centrosome or the mitotic spindle pole. We found that *Mcph1*-del neuroprogenitors contain multiple spindle poles, chromosome misalignment, and lagging chromosomes [23]. Moreover, a deletion of *Mcph1* shortens the G2 phase leading to premature mitotic entry. An uncoupled cell cycle from the centrosome cycle eventually results in switching the division mode of the neuroprogenitors from symmetric to asymmetric division [23]. Mechanistically, the localization of Chk1 to the centrosomes is abrogated in *Mcph1*-del progenitors, causing a premature Cdk1 activation and thereby a premature mitotic entry [23]. Consistent with the fact that the expression of CHK1 and BRCA1 is not affected in human MCPH1 patient cell lines [42], the *Mcph1* knockout affects neither the expression of Chk1 nor the phosphorylation of Chk1 upon DNA damage [23,49]. It is interesting to note that the ectopic expression of Chk1 generated by crossing *Mcph1*-del mice with Super-*Chk1* mice [50] failed to correct the microcephaly phenotype in our *Mcph1*-del mice (**Fig. 2**), suggesting that MCPH1 may regulate the Cdc25-Cdk1-mediated mitotic entry through a pathway independent of Chk1. Finally, consistent with its role in DDR, *Mcph1*-del neuroprogenitors are hypersensitive to IR, which is associated with a massive apoptosis in the *Mcph1*-del neocortex and an increased embryonic lethality, likely due to compromised DNA repair and increased genome instability [49]. These data indicate a DDR role for MCPH1 in the manifestation of microcephaly.

MCPH1 and Gonad Development

In addition to a smaller brain, infertility has often been observed in *Mcph1*-deficient mice. Both *Mcph1*-del mice in our lab and *Mcph1*$^{-/-}$ mice were infertile [23,36]. The testis of *Mcph1*$^{-/-}$ mice are much smaller with thinner testicular tubes and fewer spermatocytes, compared to wild-type control mice [36]. It was found that the recruitment of the DNA repair proteins BRCA2-Rad51 is impaired in mutant spermatocytes, leading to a failure of chromosomal synapsis, meiosis arrest, and apoptosis. The female *Mcph1*$^{-/-}$ mice are also infertile and harbor small ovaries without ovarian follicles [36]. These analyses indicate that the function of MCPH1 in DNA damage repair is responsible for the infertility phonotype. Consistently, we also found testis atrophy and lack of ovaries in our *Mcph1*-del mice (our unpublished observation). However, so far there is no case report on human primary microcephaly associated with infertility, or testicular or ovarian atrophy. This raises the interesting question whether the positive selection of MCPH1 during primate and human lineage evolution is closely linked to brain size expansion and the germline fitness.

MCPH1 and Tumorigenesis

As a neurodevelopmental gene, the role of *MCPH1* in brain development has been intensively studied. Interestingly, in the past few years, many studies have explored the role of MCPH1 in tumorigenesis. In humans, *MCPH1* is located on the chromosome 8p23.1, a chromosomal region frequently deleted in many types of cancers, including the breast and ovary cancers, endometrial cancer, glioblastoma and prostate cancers. The comparative genomic hybridization assay shows that 38/54 (72%) breast cancer cell lines contain a decreased copy number of *MCPH1* [31]. The MCPH1 mRNA level is markedly decreased in 19/30 (63%) of ovarian cancer specimens compared to benign ovarian tissue [31]. The MCPH1 mRNA and protein levels were found to be down-regulated in 21/41 (51.22%) and in 19/25 (76%) oral squamous cell carcinoma samples, respectively [51]. Immunohistochemistry (IHC) studies revealed a down-regulation of MCPH1 in 7/10 (70%) breast cancer biopsies [52] and in 93/319 (29%) breast cancer cell lines [53]. The *MCPH1* copy number and the protein level [54] are markedly reduced in advanced epithelial ovarian cancer as revealed by IHC. Moreover, a decreased MCPH1 expression is observed in 19 of 31 cases (61.3%, at the mRNA level) and in 44 of 63 cases (69.8%, at the protein level) of cervical cancer [37]. A recurrent heterozygous mutation (c.904_916del, p.Arg304ValfsTer3) in MCPH1 was identified to be significantly associated with breast cancer susceptibility both in familial (5/145, 3.4%, $P = 0.003$, OR 8.3) and unselected cases (16/1150, 1.4%, $P = 0.016$, OR 3.3) [55]. MCPH1 alterations have been reported in many malignant tumors, including breast cancer (~7%), prostate cancer (16%), bladder urothelial carcinoma (11%), lung cancer (9%), ovarian cancer (8%), liver cancer (7%), and colorectal adenocarcinoma (9%). A detailed list of all MCPH1 alterations in tumors can be found in The Cancer Genome Atlas (TCGA;

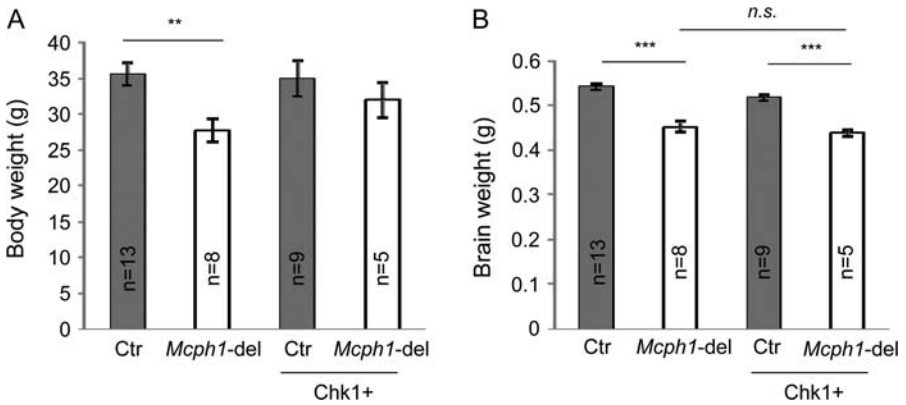

Figure 2. The extra copy of Chk1 has no impact on the brain size of *Mcph1*-del mice (A) The body weight of adult animals from the indicated genotypes (Chk1−: 6–12 months; Chk1+: 3–9 months). (B) The brain weight of adult animals from Student's *t*-test was performed for statistical analysis. $^{**}P < 0.01$; $^{***}P < 0.001$; n.s., not significant.

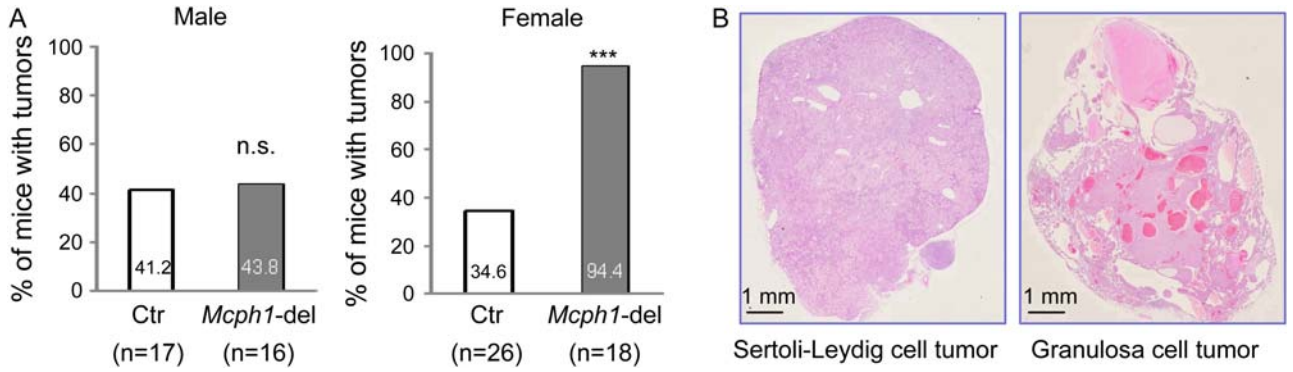

Figure 3. *Mcph1*-del female mice develop a high incidence of ovarian tumors in a period of 1.5–2.5 years (A) The percentages of male mice and female mice with tumors are shown. The statistical analysis was performed by a χ^2 test. (B) A histological analysis of the ovarian tumors in *Mcph1*-del mice reveals granulosa cell tumors and Sertoli-Leydig cell tumors. H&E staining of tumor sections. ***$P < 0.0001$; n.s., not significant.

cBioPortal Cancer Genomics database, http://cancergenome.nih.gov/). Conversely, an overexpression of MCPH1 inhibits the migration and invasion of cervical cancer cells by inducing the S-phase arrest and apoptosis [37]. These studies suggest that MCPH1 is a tumor suppressor.

Mcph1-knockout mouse studies also support the tumor suppressor role of MCPH1. It has been shown that 17.1% of the $Mcph1^{-/-}$ mice develop malignant tumors, originated from lymphomas and granulosa ovary tumors, in contrast to 8.9% in $Mcph1^{+/-}$ and 5.3% in wild-type mice over a period of 2.5 years [43]. Moreover, a specific *Mcph1* knockout in mouse mammary glands results in breast cancer triggered by a low dose of IR [22]. A deficiency of MCPH1 accelerates the lymphoma development in a $p53^{-/-}$ background [43]. $Mcph1^{-/-}p53^{-/-}$ cells are hypersensitive to IR, which is likely due to a compromised DNA repair by both HR and non-homologous end joining [43]. Consistent with this notion, *MCPH1* deficiency enhances chromosomal aberrations, aneuploidy as well as abnormal centrosome multiplication in $p53^{-/-}$ MEF cells. In this regard, it is interesting to note that MCPH1 enhances p53 protein stability and activity by blocking the MDM2 (murine double minute 2)-mediated p53 ubiquitination and degradation [56]. Thus, the genome instability caused by a loss of MCPH1 contributes to tumorigenesis. We also observed a high incidence (94.4%) of ovary tumors (granulosa cell tumor and Sertoli-Leydig cell tumor) in *Mcph1*-del mice over a period of 1.5–2.5 years (**Fig. 3**). However, we did not observe malignancies in other organs. The discrepancy of tumor types in these two mouse models ([43] and our observation) may be explained by the nature of the mutation introduced to disrupt the protein domains, which would target different cell types.

Conclusion and Perspective

MCPH1 is the first identified causal gene of the human neurodevelopmental disorder, autosomal recessive primary microcephaly. Intensive molecular and cellular studies show that MCPH1 plays a pleiotropic role in DDR, cell cycle control, chromosome condensation, and apoptosis. These functions establish MCPH1 as an important player in brain development, fertility, as well as in tumorigenesis. How MCPH1 itself is regulated remains largely unknown. The miR-27 expression is negatively correlated with the MCPH1 protein level in renal cancer and miR-27 negatively regulates the MCPH1 expression through the 3′-UTR of MCPH1 [51,57]. Moreover, MCPH1 was reported to interact with Cdc27, a scaffold component of the ubiquitin E3 ligase APC/C (anaphase-promoting complex/cyclosome) [58]. Interestingly, the *Drosophila* MCPH1-B isoform undergoes a robust degradation in the *Xenopus* interphase egg extraction supplemented with Cdh1 [32], suggesting that the MCPH1 turnover is tightly regulated during cell cycle progression. The significance of the turnover of the MCPH1 protein in cellular activity and in the homeostasis of the brain and other organs presents a new research topic in the future.

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