

REVIEW ARTICLE

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STIL balancing primary microcephaly and cancer

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Abstract

Cell division and differentiation are two fundamental physiological processes that need to be tightly balanced to achieve harmonious development of an organ or a tissue without jeopardizing its homeostasis. The role played by the centriolar protein STIL is highly illustrative of this balance at different stages of life as deregulation of the human *STIL* gene expression has been associated with either insufficient brain development (primary microcephaly) or cancer, two conditions resulting from perturbations in cell cycle and chromosomal segregation. This review describes the recent advances on STIL functions in the control of centriole duplication and mitotic spindle integrity, and discusses how pathological perturbations of its finely tuned expression result in chromosomal instability in both embryonic and postnatal situations, highlighting the concept that common key factors are involved in developmental steps and tissue homeostasis.

Facts

- STIL is a cell cycle-regulated protein specifically recruited at the mitotic centrosome to promote the duplication of centrioles in dividing cells.
- Complete loss of STIL results in no centrosomes, no cilia, and is not compatible with life.
- By contrast, residual or increased expression of STIL is viable but alters the centriole duplication process leading to either impaired or excessive centrosome formation.
- Genetic mutations in human STIL result in either residual expression or stabilization of STIL at the centrosome both leading to mitotic spindle defects and primary microcephaly (MCPH7).
- Abnormally high expression of STIL in differentiated tissues triggers centrosomal amplification and is associated with an increased metastatic potential in multiple cancers.

Open questions

- Centrosome amplification is seen both in cancer and in MCPH phenotype. How is the context important in determining the phenotype?
- Is presence of STIL in the centrosome important in determining cell fate?
- STIL has several binding partners. To what extent is the STIL phenotype due to the independent functions of these binding partners?

Introduction

The developing brain appears particularly sensitive to centrosome dysfunction, which is also associated with a wide range of cancers. The centrosome is a cytoplasmic organelle built around microtubule-based core components called centrioles. This review focuses on the *STIL* gene that encodes a regulatory protein necessary for centriole biogenesis and is expressed in many cell types. The structure and function of STIL is described followed by an account of two phenotypes that have been associated with STIL dysfunction, autosomal recessive primary microcephaly (MCPH), and cancer. Reasons for these different phenotypes are discussed in relation with

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Edited by A. Verkhratsky

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centriole duplication and mitotic checkpoints that operate in different cellular contexts to deal with aneuploidy and chromosomal instability.

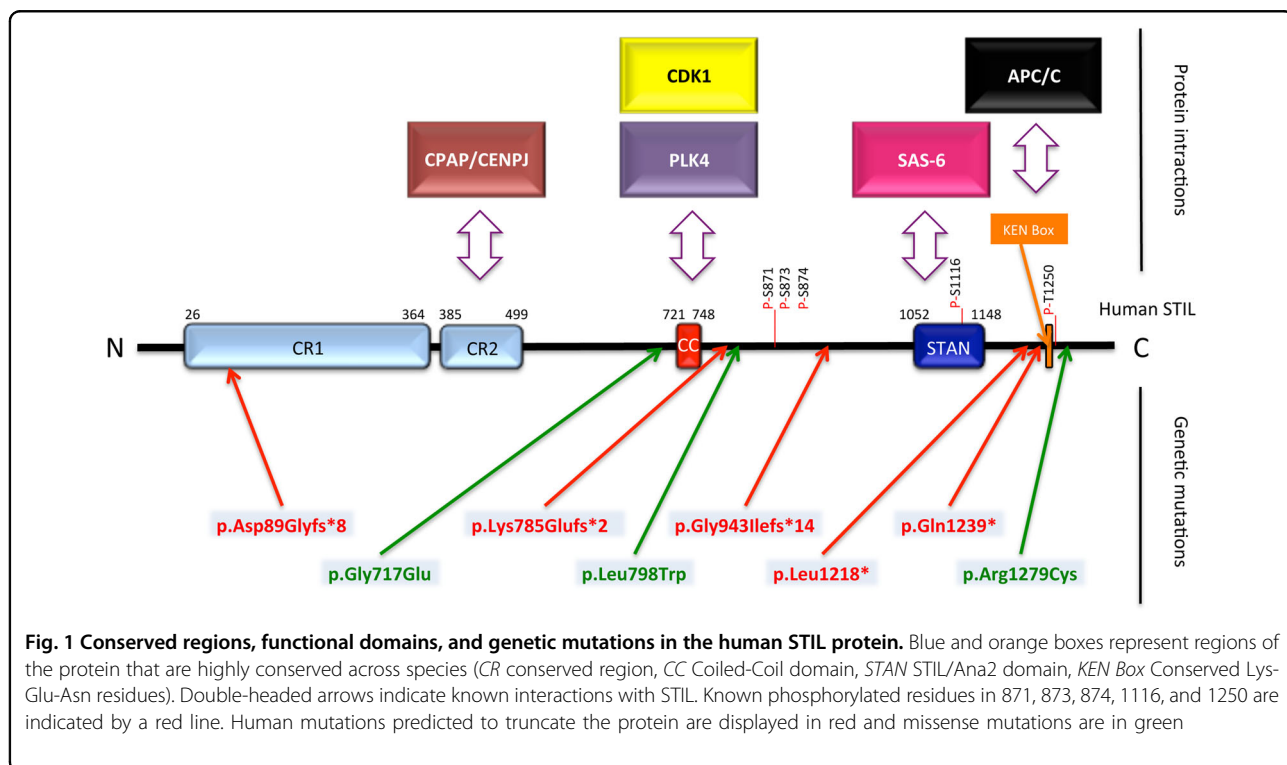
STIL structure

The human *STIL* gene was initially identified in a common chromosomal rearrangement in T-cell acute lymphoblastic leukemia and named *SCL/TAL1* Interrupting Locus (*SIL/STIL*)¹. It is predicted to encode five isoforms. Isoform1 (NP_001041631.1; henceforth referred to as *STIL*) is a 1288-amino-acid protein; isoform2 has 1287 amino acids (NP_001269865.1) and differs from isoform1 by a missing serine875; isoforms3–5 (NP_001269866.1, NP_001269867.1, NP_001269868.1) have several amino acids missing but their significance is unknown. *STIL* contains conserved regions and interacts with several proteins (Fig. 1). CR2 (amino acids 385–499) is a proline-rich domain that includes the conserved PRXXPP motif, which interacts with the Centrosomal P4.1-Associated Protein (CPAP/CENPJ). The coiled-coil domain (amino acids 721–748) is important both for Polo-Like Kinase 4 (PLK4) and Cyclin Dependent Kinase 1 (CDK1 also known as CDC2)/CyclinB binding^{2,3} and for *STIL* oligomerization⁴. The STAN domain (amino acids 1052–1148) mediates the binding of the centriole protein SAS-6². At the C terminus is the KEN box involved in Anaphase-Promoting-Complex/Cyclosome (APC/C)-mediated degradation of *STIL*⁵. The C terminus of *STIL*

can also interact with conserved components of the Hedgehog signaling such as Suppressor-of-fused homolog (SUFU)⁶ and GLI1⁷.

STIL function in centriole duplication

The centriole is an evolutionarily conserved structure consisting of a ninefold symmetric cartwheel with microtubule triplets, and thought to have been present in the Last Eukaryotic Common Ancestor⁸. The centrosome has two centrioles orthogonal to each other, surrounded by proteins constituting the pericentriolar material (PCM). Both the centriolar core and the PCM nucleate microtubules, which are important for positioning the mitotic spindles and imparting polarity and asymmetry to the cell. During cell division the centrioles duplicate only once; *STIL* and its interactors are central to this process, ensuring the fidelity of this unique duplication, thereby minimizing chromosome instability⁹. *STIL* transiently associates with PLK4 and SAS-6 to form the core module for centriole duplication¹⁰. PLK4 is related to the Polo-kinase family of serine/threonine kinases and initiates centriole formation^{11,12}, while SAS-6, a coiled-coil protein that self-assembles into the ninefold symmetry gives the cartwheel its shape^{13,14}. *STIL* and PLK4 are initially maintained at low levels in non-dividing and differentiated cells¹⁵; *STIL* forms dimers or tetramers through its CC domain and is degraded in the cytoplasm by APC/C and its co-activator CDC20, through recognition of the KEN motif^{4,5}. *STIL*



level in the cytoplasm rises in G1 because its degradation by APC/C is prevented by the absence of CDC20⁵. PLK4 recruits STIL at the base of the parental centriole through the CC domain and phosphorylates the STAN domain at the late G1/G1-S transition^{16–18}. Phosphorylated STIL then promotes its binding to the C-terminal region of SAS-6 and recruits it to the outside wall of the centriole^{2,17}. This core module along with CPAP, also recruited by STIL to the centriole, starts the assembly of the cartwheel and centriole duplication^{19,20}. At early S phase, the centrosome-associated protein ROTATIN was recently shown to associate with STIL and contribute to building full-length centrioles²¹. At S–G2 transition, the centrioles are duplicated but remain close to each other, potentially preventing reduplication. Remarkably, CDK1, transiently expressed at late G2 and during M phase, competes with PLK4 for binding to the CC domain of STIL, preventing PLK4 from recruiting STIL until mitosis is completed^{3,16,22}. The spindle assembly checkpoint, a highly conserved mechanism also called mitotic checkpoint, prevents the degradation of CDK1 during mitosis by the inhibition of APC/C–CDC20 until all chromosomes are properly segregated²³. CDK1 is thought to trigger progressive dissociation of STIL and SAS-6 from early mitotic centrosomes, thereby initiating cartwheel disassembly during M phase and ensuring that centriole biogenesis occurs only once before cell divides⁵. Upon activation, APC/C–CDC20 degrades CDK1 for entry into anaphase and again marks STIL for degradation until next the G1 phase. Thus, by prometaphase there is no STIL at the centrosome and by anaphase there is no STIL in the cytoplasm either until upon mitotic exit APC/C is blocked and STIL levels builds up once again in G1 (Fig. 2).

STIL function in SHH signal transduction

SHH is a morphogen involved in patterning, proliferation, and survival of neural stem cells during development²⁴. SHH binds to its receptor Patched thereby relieving its inhibition on Smoothened and resulting in the activation of GLI transcription factors. That GLI proteins are the main downstream effectors for SHH signaling is borne out by the fact that activated GLI can rescue the loss-of-function of SHH and lead to proliferation and increased cell survival²⁵. The initial findings that STIL participates in the control of SHH signaling came from *Stil*^{−/−} mouse embryos, which showed a marked reduction of Patched and Gli1 expression²⁶. Interestingly, *Stil*^{−/−} mutants also lack primary cilia²⁷, a structure present in almost all cell types, which is assembled beneath the plasma membrane, by the protrusion of the microtubule-based axoneme. During interphase, the mother centriole and its appendages constitute the basal body, from which the axoneme elongates^{28,29}. STIL requirement for cilia formation likely stems from its function in supporting centriole biogenesis and stability.

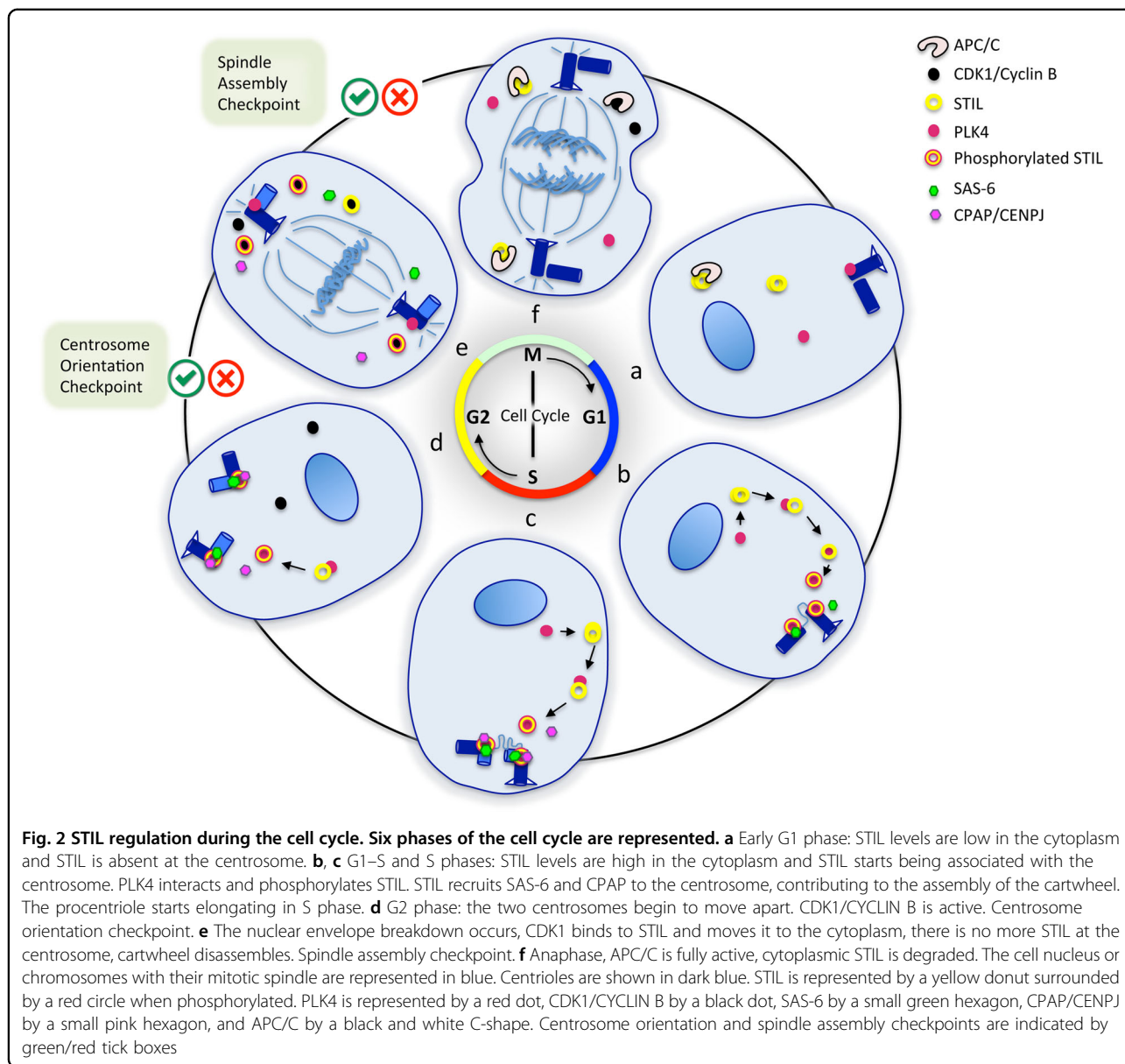
Receptors for SHH are abundant on cilia membranes allowing cilia to act as receivers of signals and as platforms where downstream effectors can be modified³⁰. Thus, cilia, whose presence depends on STIL, are required for activity of the SHH pathway³¹.

STIL also interferes with the SHH pathway in a direct manner, by interacting with SUFU. SUFU acts as a negative regulator of SHH by tethering GLI1 in the cytoplasm. STIL binds to SUFU in the cytoplasm of pancreatic cancer cells, thus releasing GLI1 for transcription of SHH-downstream genes⁶. In PC12 cells, STIL interacts with the SUFU/GLI1 complex, and its down-regulation results in a decrease in both SHH signaling and cell proliferation⁷. A similar correlation has been evidenced in zebrafish retina cells, further suggesting that STIL plays a role in cell proliferation through the SHH pathway³² (Fig. 3).

Consequences of STIL deregulation

Given the centrality of STIL in centrosome duplication and cilium biogenesis, deregulation of STIL is predictably profound. *Stil*^{−/−} embryos die at midgestation with axial midline defects, resulting from aberrant SHH signaling²⁶. MEFs derived from *Stil*^{−/−} embryos show a marked decrease in mitotic index, and *Stil* knockdown results in the absence of identifiable centrosomes during interphase and multiple spindle poles with disrupted γ -tubulin signals during mitosis³³, as well as in the loss of primary cilia²⁷. Conversely, STIL overexpression causes centrosome amplification, resulting in a star-like pattern around the parental centriole, consistent with a role of STIL in the onset of procentriole formation. Accordingly, this star-like structure is positive for the centriolar proteins CP110 and centrin^{20,34}. Mutations within the various domains of STIL give predictable phenotypes. When the CR2 domain or the CC domain or the STAN domain is deleted, there is no centrosomal duplication^{16,18}, whereas removal of the KEN box leads to centrosome amplification due to the inability of STIL to be degraded⁵. However, both overduplication and lack of duplication of centrosomes lead to abnormal mitotic spindle assembly and consequently increase the chances of abnormal chromosomal segregation and aneuploidy.

Another consequence of STIL deregulation is the indirect effect on interacting partners. STIL binding to PLK4 suppresses PLK4 auto-inhibition, thereby allowing its trans-phosphorylation and protecting activated PLK4 from degradation³⁵. Conversely, depletion of STIL leads to a marked accumulation of PLK4 in an inactive conformation. Therefore, changes in STIL levels have immediate consequences on PLK4 activation levels^{10,17}. STIL also negatively regulates Chfr, an E3 ligase that blocks mitotic entry in response to mitotic stress.

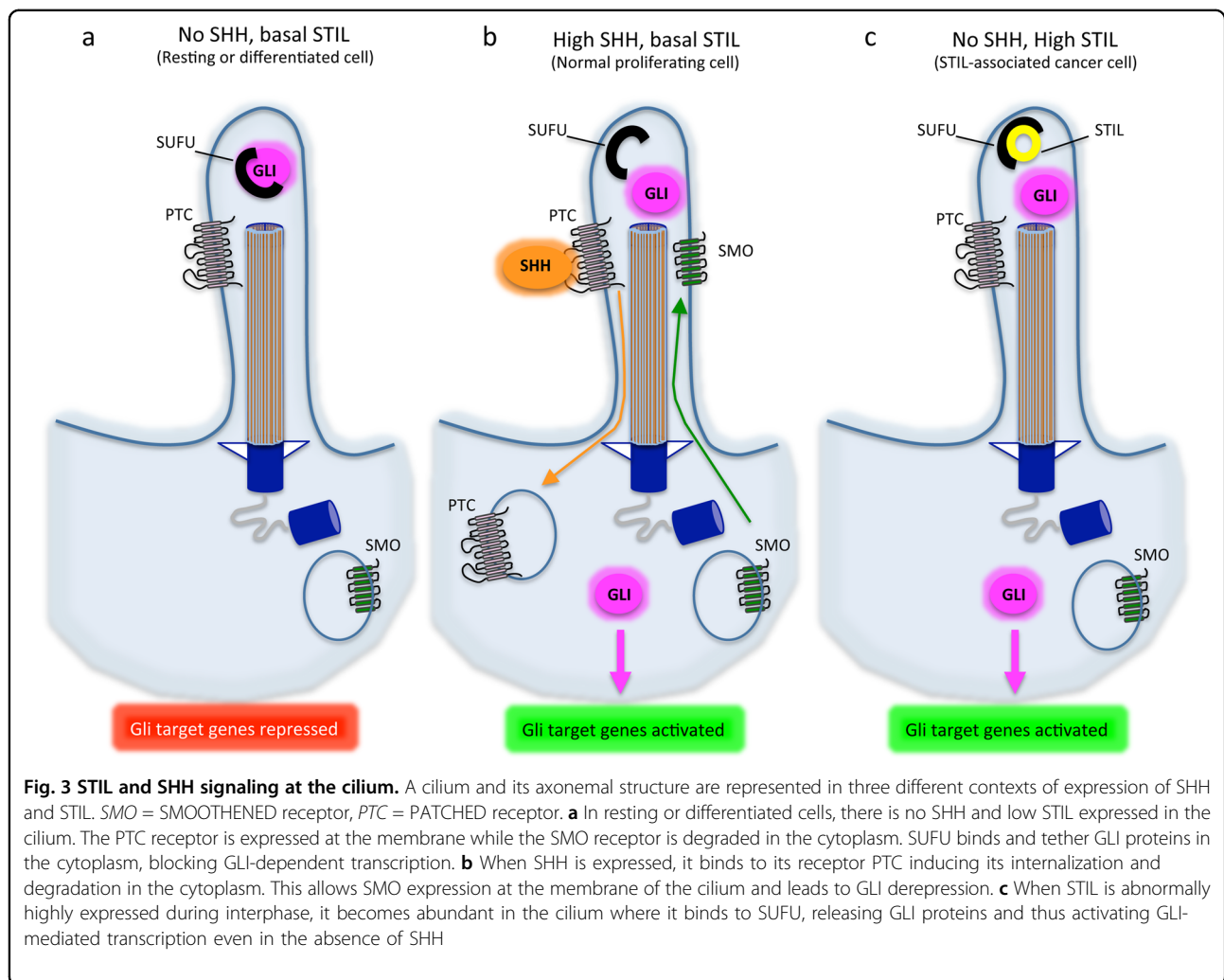


No direct interaction between STIL and Chfr has been shown, but STIL expression results in an increase of Chfr auto-ubiquitination, thereby promoting its proteasomal degradation and proper progression of cells through mitosis³³. Data support a role of Chfr in defective mitotic progression associated with reduced activation of CDK1/CYCLIN B and centrosomal abnormalities caused by the lack of STIL³³. Thus, STIL deregulation likely impacts the activation level of CDK1/CYCLIN B and the timing of entry into mitosis, through its regulation of Chfr.

STIL expression during human brain development

STIL is expressed in both fetal and adult tissues. However, its expression levels fluctuate with the cell cycle,

making it difficult to detect in whole tissue, especially if the cells are not synchronized. Indeed, it is detected more easily in cancer cell lines and tissue where it is over-expressed^{36,37}. The BrainSpan Atlas (<http://www.brainspan.org/>) and the BrainCloud database (<http://braincloud.jhmi.edu>) show its expression pattern during brain development. The association of STIL with cell proliferation is borne out by its expression pattern during fetal stages. At 15 postconceptional weeks STIL is strongly expressed in the ventricular and subventricular zones of the forebrain, the ganglionic eminence and the rostral migratory stream, and less expressed in intermediate zone, subplate, cortical plate, marginal zone, and subgranular layer of the forebrain. This pattern persists at 21 postconceptional weeks, although the expression is



reduced in the subventricular zone. The expression of *PLK4*, *SAS-6*, and *CPAP* also broadly shows this pattern of expression, although the expression of *SAS-6* and *STIL* diverges in some regions of the cortical plate. In the cerebellum, *STIL*, *PLK4*, and *SAS-6* but not *CPAP* are expressed in the external granule layer and regions of the rhombic lip. However, none of these genes are expressed in the transient Purkinje cell cluster, the ventricular matrix zone of the cerebellum or in the migratory streams of the hindbrain. Microdissection experiments at different developmental stages confirmed that *STIL* expression is high in the ventricular zone and low in the subplate and cortical zones (<http://www.brainspan.org/>). Further, microdissected diencephalon did not have *STIL* (<http://www.blueprintnhpatlas.org/>). This suggests that (i) the structural components of the cartwheel *STIL*-*SAS-6*-*PLK4* is by no means obligatory for centrosome duplication in all cells and (ii) *STIL* is present in specific populations of dividing cells.

STIL mutations and primary microcephaly

Microcephaly (small brain size) is indirectly diagnosed by a head circumference smaller than the age-specific and gender-adjusted mean by more than 2 standard deviations (S.D.s) at birth. Primary microcephaly refers to hereditary microcephalies already detectable *in utero*. Most of them are autosomal recessive and include (i) isolated forms called MicroCephal Primary Hereditary (MCPH), (ii) forms associated with growth retardation, called microcephalic dwarfism. Most of *STIL* mutations identified in patients are associated with a MCPH phenotype and *STIL* is recognized as MCPH7^{38–40}. However, a few patients exhibit criteria of microcephalic dwarfism, as short stature has been reported⁴¹. So far, eight mutations in *STIL* have been described in 37 patients all showing severe microcephaly (−4 to −10 S.D.). Five mutations are splice^{42,43}, deletion, nonsense⁴³, or duplication⁴⁴ that predict the production of truncated proteins, while three mutations are missense substitutions^{44–46} (Fig. 1). Despite the different

Table 1 List of mutations identified in the human STIL gene, and their functional consequence

Mutation (nt)	Mutation (aa)	Domain affected (protein)	Functional consequence	Head OFC (S.D.)	Brain MRI data	References
c.453+5G>A (splice donor)	p.Asp89Glyfs*8 (truncating)	All domains	No functional protein	-9 to -10	Short cc, simplified gyration, lobar HPE	42
c.2150G>A	p.Gly717Glu (missense)	CC domain	Presumably inability of STIL to oligomerize and interact with PLK4—decreased centriole duplication capacity	-7 to -8	Partial cc agenesis, simplified gyration, lobar HPE	4,45
c.2354_2355dupGA	p.Lys785Glyfs*2 (truncating)	Phosphorylation sites, STAN domain, KEN box	Removes critical PLK4 phosphorylation sites	26 cm at birth	Cc agenesis, simplified gyration, lobar HPE	44
c.2392 T > G	p.Leu798Trp (missense)	—	ND	NA	NA	46
c.2826+1G>A (splice donor)	p.Gly943Ilefs*14 (likely truncating)	STAN domain, KEN box	Not able to duplicate centrosomes since they cannot recruit SAS6	-5	NA	27,43
c.3655delG	p.Val1219* (initially reported as p.Leu128*; truncating)	KEN box	Resistance to APC/C-mediated degradation	-4 to -10	NA	5,18,27,43
c.3715 C > T	p.Gln1239* (truncating)	KEN box	Resistance to APC/C-mediated degradation	-7 to -8	NA	18,27,43,95
c.3835 C > T	p.Arg1279Cys (missense)	C terminus	ND	26 cm at birth	Cc agenesis, simplified gyration, lobar HPE	44

kinds of mutations, the various domains affected (Table 1), and the report of lobar holoprosencephaly and partial agenesis of the corpus callosum in addition to microcephaly in some cases^{42,44,45}, no clear genotype–phenotype correlation has come out so far. However, the fact that these mutations trigger a phenotype compatible with life (although very severe) is surprising since genetic ablation of *STIL* is embryonically lethal early during embryogenesis in mice and fish^{26,47}. This suggests that *STIL* mutations in human do not result in a complete loss-of-function, or are partially compensated by other genes. The first hypothesis is supported by the findings that two C-terminal nonsense mutations (p.Val1219* and p.Gln1239*), which result in the loss of the KEN box⁴³, do not affect the centrosomal localization of *STIL* nor its functionality but rather abolish its degradation by APC/C in late mitosis⁵. Removal of the KEN box thus causes a strong accumulation of the mutant protein, resulting in centrosome amplification⁵. Alternatively, microcephaly in MCPH7 patients can result from a decrease of *STIL* levels. This was illustrated by rescue experiments in U2OS cells showing that the pGly717Glu mutation induces a reduced but non-null activity of *STIL* on centriole duplication⁴⁵. Another mutation, inducing exon 5 skipping and frameshift in the *STIL* sequence, is

likely close to a null mutation as all domains are predicted to be lost⁴². However exon skipping was only partial in the patient analyzed, suggesting some residual activity of *STIL*, and providing an explanation for the fact that this mutation is compatible with life and the idea that MCPH7 microcephaly can result from a decreased *STIL* activity. Therefore, both accumulation and impairment of *STIL* protein levels during cell cycle affect centriole regulation and result in microcephaly (Fig. 4).

STIL deregulation and cancer

Centrosomes are essential for chromosomal stability, and abnormalities of their number, or structure affect cell division²². Considering the central role of *STIL* in maintaining centrosome integrity in highly proliferating cells, *STIL* has been found upregulated in several cancers of bad prognosis, including lung cancer, colon carcinoma, prostate adenocarcinoma³⁶, and ovarian cancers⁴⁸. Moreover, *STIL* expression is associated with an increased metastatic potential in multiple cancers⁴⁹. As expected, *STIL* upregulation is associated with a high histopathological mitotic index in tumors³⁶ and presumably affects the formation of mitotic spindles, as well as SHH signaling and the function of its interactors.

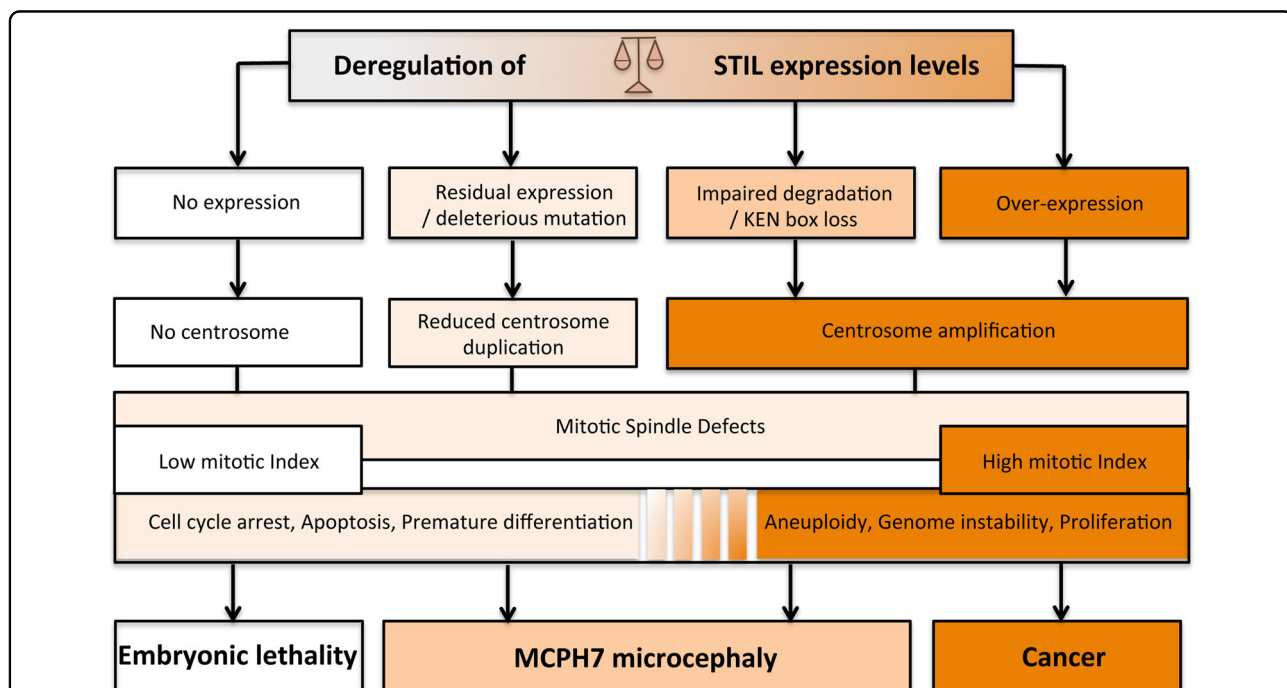


Fig. 4 Consequences of deregulating *STIL* expression levels. *STIL* expression levels are finely regulated both during development and in differentiated tissues. While total absence of *STIL* is lethal during development, high *STIL* levels due to random somatic mutations in differentiated tissues result in aneuploidy and/or high GLI-mediated transcription that can lead to cancer. Congenital deregulation of *STIL* (both a too high and a too weak expression) leads to microcephaly by either reduced or amplified centrosome duplication, highlighting the importance of a tightly regulated *STIL* expression

Although cells can proliferate and microtubule can nucleate in their absence, centrosomes are obligatory for controlling spindle orientation⁵⁰. Loss of this control leads to spindle defects, especially in highly polarized cells, where misoriented spindles influence daughter cell positioning, possibly causing a disruption in tissue morphology⁵¹. Several tumor suppressor genes such as *APC*, or *VHL*, are important for stabilizing microtubules and maintaining spindle orientation⁵¹. Similarly, overexpression of genes such as *STIL* could act as oncogenes and lead to cancer by promoting spindle defects, although direct evidence of a link between oncogene activation and spindle misorientation is lacking. *STIL* overexpression, which results in supernumerary centrosomes, could lead to cancer in inducing chromosomal instability^{10,52}.

The role of *STIL* in promoting SHH signaling could be another pathway to account for its association with cancer. Following the initial observation that SHH overexpression results in the development of basal cell carcinomas in mice⁵³, a large body of literature has implicated the SHH pathway in development of various cancers including a subset of medulloblastomas^{54,55}. *STIL* overexpression in pancreatic adenocarcinoma was shown to de-repress *GLI1* from *SUFU*-mediated control and this phenotype was reversed when *STIL* was downregulated⁶. Thus, during the process of carcinogenesis, the increased expression of *STIL* promotes the transcriptional activity of *GLI1*, which is no longer regulated quantitatively. *GLI1* upregulates genes that promote sustained proliferation, cell death resistance, stemness, angiogenesis, and genomic instability, which are hallmarks of cancer⁵⁶. Therefore, increase in *STIL* expression leading to uncontrolled *GLI1* de-repression likely represents a crucial step toward cancer progression.

MCPH and cancer—two conditions associated with abnormal centrosome duplication

STIL loss-of-function results in embryonic lethality in mice, fish, and most likely in human as well. MCPH7 phenotype reflects that perturbing the tuning of *STIL* expression levels is compatible with life but triggers severe patterning phenotypes during development, including brain growth. Thus, microcephaly caused by *STIL* mutations can result from either too less centrosome duplication or too much centrosome amplification (Fig. 4). In both cases, the integrity of the mitotic spindle is compromised and cell divisions have a greater chance of chromosomal instability. Similarly, the association of *STIL* upregulation with various cancers reflects a deregulation of *STIL* expression levels in specific tissues contributing to genomic instability. What results in MCPH rather than cancer could be the difference in mitotic checkpoints in different contexts^{13,57}. Centrosome orientation checkpoint ensures that the centrosomes are duplicated and

appropriately positioned before entry into mitosis and several centrosomal proteins such as CNN and SAS-6 are part of this checkpoint⁵⁸. This checkpoint may not be present in all cells at all developmental stages but rather in those cases where the cell division plane has important outcomes⁵⁹. In germline stem cells, for example, the division plane determines the distribution of cell fate determinants in daughter cells, holding cells at the G2–M phase until their mitotic spindles are properly oriented⁵⁸. *STIL* downregulation results in disorganized mitotic spindles resulting in a loss of spindle orientation control⁴⁷. In cortical development, *STIL* mutations leading to spindle orientation defects likely result in a depletion of cortical progenitors due to cell death or premature differentiation and give rise to a MCPH phenotype⁵⁷ (Fig. 4). *PLK4* overexpression also results in centrosome amplification and aneuploidy leading to a reduction in brain size due to cell death⁶⁰. Apoptosis inhibition in this background causes the accumulation of aneuploid cells unable to proliferate efficiently, leading to premature neuronal differentiation⁶⁰, whereas in a *P53*^{-/-} context, *PLK4* overexpression results in aneuploidy and skin cancer⁶¹.

The spindle assembly checkpoint is the next checkpoint once cells have entered mitosis and formed spindles. In the absence of bipolar spindles, cells are held-up in prometaphase and do not proceed to anaphase⁵⁸, which results in increased apoptosis⁶². However, cells can increase their time in mitosis, thus adapting to this checkpoint and can assemble bipolar spindles in the absence of centrosomes or in the presence of supernumerary centrosomes. First in the absence of centrosome duplication cells manage to have bipolar spindles even though there is no centriole attached to one of the poles³⁴. Second is a phenomena of centrosome clustering, wherein multiple centrosomes are clustered to enable the formation of bipolar spindles, a phenomenon whose efficiency could be tissue-specific⁶⁰. These divisions often result in aneuploidy. When centrosome integrity is compromised, cells usually arrest in G1 and do not re-enter cell cycle after a prolonged cytokinesis to avoid chromosomal instability²² and in many cases proliferation in the absence of centrioles additionally requires the suppression of *P53*¹⁷. Upregulation of *STIL* giving rise to aneuploidy could play a major role in providing an evolving genome to help the cells adapt to the changing environment of the cancer and escape normal checkpoints. In the developing cortex, abnormal mitotic spindles due to *STIL* mutations would result in cell fate switches rather than cell hyperproliferation. However, that patients whose *STIL* mutation is clearly associated with centrosome amplification could be at risk of developing cancer cannot be ruled out.

Seventeen MCPH loci have been described so far⁴⁰, several of which also play a role in cancer. MCPH1 is an early DNA damage response (DDR) protein and *MCPH1*

deletions have been identified as a risk for breast cancer⁶³. Centrosome amplification is commonly seen following DNA damage and can arise as part of the DDR⁶⁴. The serine-threonine kinase CHK1 plays a major role in DDR by halting cell division in G2/M until repair proteins are recruited at lesion sites. In this context, MCPH1 deficiency potentiates CHK1 activity and increases centrosome amplification⁶⁵. Thus, compromising mitotic checkpoints results in cell division in the presence of abnormal centrosomes^{63,65}. How CHK1 overactivation leads to centrosome amplification is not fully understood, but it is thought to activate CDK2, a cyclin-dependent kinase required for centrosome duplication, likely through activating its phosphorylation^{66,67}. WD repeat-containing protein62 (WDR62), whose deficiency is associated with MCPH2^{68–70}, is required for maintaining spindle and centrosome integrity. Its overexpression, coincident with centrosome amplification, is also seen in lung adenocarcinomas and ovarian cancers^{71,72}. WDR62 interacts physically during the cell cycle with the abnormal spindle-like microcephaly-associated protein (ASPM), whose deficiency causes the most frequent primary microcephaly (MCPH5)⁷³. Here too, increased ASPM levels cause tumor growth and are seen in medulloblastomas while its reduction causes a decrease in tumor proliferation⁷⁴. CDK5RAP2 (MCPH3) is also involved in the DDR; it functions by arresting cells before mitotic entry due to impaired centrosomes but also interacts with BUB1 and MAD2, which are important in spindle activation checkpoint^{75,76}. Interestingly, a correlation between centrosomal abnormalities, aneuploidy, and cytogenic risk profile is seen in acute myeloid leukemia, where gene expression profiling has revealed the differential expression of genes encoding centrosomal and mitotic spindle proteins⁷⁷. Among these are pericentrin, a scaffold protein that anchors many other proteins at the centrosome⁷⁸ and NuMA, which associates with dynein and microtubules to create localized pulling forces, thus regulating the correct assembly and positioning of the mitotic spindle⁷⁹. The role of such factors points to mitotic centrosomal abnormalities as important component of cancer progression.

STIL could also have an indirect effect on cancer, as a downstream effector of PLK4. Increased PLK4 expression has been reported in malignancies such as colorectal cancer⁸⁰, pediatric medulloblastoma⁸¹, and breast tumors⁸². Its transient overexpression leads to centrosome overduplication and in a P53^{-/-} background that inhibits apoptosis, it results in aneuploidy and spontaneous skin cancer⁶¹. Another recent study has convincingly shown that PLK4 overexpression leads to spontaneous tumors in several organs⁸³. It remains to be seen whether this phenotype is dependent on STIL expression. STIL binds to PLK4 in the cytoplasm and

therefore STIL expression levels could impact PLK4 cytoplasmic activity¹⁷, which functions to remodel the cytoskeleton and may be important for cancer invasion and metastasis as its depletion is correlated with an increase in E-cadherin expression and less metastasis⁸⁴. CYCLIN B is also frequently found elevated in primary breast cancer, esophageal squamous cell carcinoma, laryngeal squamous cell carcinoma, and colorectal carcinoma^{85–89} and its expression indicates a bad prognosis and is correlated with the malignancy of gynecological cancers⁹⁰. Downregulation of *STIL* decreases CDK1/CYCLIN B activity, prevents G2–M transition, and causes inhibition of tumor growth in vivo⁹¹. Conversely, increasing STIL could promote CDK1/CYCLIN B activity and indirectly participate in CYCLIN B-dependent proliferation in tumor cells. Absence of STIL also results in an upregulation of Chfr, and lowers PLK1, resulting in the activation of the CDC25c phosphatase. This pathway could thus control the entry of cells into mitosis independent of its obligatory role in centriole duplication³³.

Conclusion

STIL mutations in MCPH show that centrosomes and cilia are essential for normal brain development. During evolution, one way that the cortex has undergone expansion is likely linked to mechanisms controlling spindle orientation and keeping the balance between asymmetric and symmetric cell divisions^{92,93}. A change in the nature of spindle orientation control has been proposed to account for the population of subventricular zone progenitors in human development and based on the strong expression of *STIL* in the ventricular zone, one may speculate that STIL is important for controlling spindle orientation in giving rise to subventricular zone's cells⁹⁴. Deficiency of several centrosomal proteins results in MCPH, and centrosomal proteins may thus be a key to discovering the mechanism of expansion of cortical area³⁸. In addition, the involvement of STIL in cancer shows that the result of spindle defects can be vastly different depending on the stage of development and the tissue involved that probably is partly due to differential responses to mitotic checkpoint mechanisms. STIL intersects several pathways, and lowering its level could block the effects of *PLK4* overexpression, CDK1/CYCLIN B activation, and GLI1 signaling, and reduce proliferation even in the presence of aneuploidy. Thus, centrosomal proteins may be a new class of targets for cancer.

Acknowledgments

This work was supported by the Institut National pour la Santé et la Recherche Médicale (INSERM), the Centre National de la Recherche Scientifique (CNRS), the Université Paris 7, the DHU PROTECT, the Indo-French Centre for the Promotion of Advanced Research "CEFIPRA" (grant no 4903-02 to S.M., V.E.G., P.G., and S.P.), and grants from the French National Research Agency (ANR-13-

RARE-0007-01 to S.P., P.G., and V.E.G. (ERA-NET E-Rare 2013, EuroMicro project), and ANR-16-CE16-0024-01 to V.E.G. (PRC GÉNÉRIQUE 2016, MicroGol project)). S.M. was supported by a grant from Curadev Pharma.

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Competing interests

The authors declare that they have no competing financial interests.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 29 August 2017 Revised: 4 October 2017 Accepted: 23 October 2017

Published online: 19 January 2018

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