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Ubiquitin in Regulation of Spindle Apparatus and its Positioning: Implications in Development and Disease

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Abstract

Emerging data implicates ubiquitination, a post-translational modification, in regulating essential cellular events -- one of them being mitosis. In this review we discuss how various E3 ligases modulate the cortical proteins, like, dynein, LGN, NuMa, Gα along with polymerization, stability, and integrity of spindles. These are responsible for regulating symmetric cell division. Some of the ubiquitin ligases regulating these proteins include PARK2, BRCA1/BARD1, MGRN1, SMURF2 and SIAH1; these play a pivotal role in the correct positioning of the spindle apparatus. A direct connection between developmental or various pathological disorders and the ubiquitination mediated cortical regulation is rather speculative, though deletions or mutations in them lead to developmental disorders and disease conditions.

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1. Introduction

One of the earliest events that occur in a cell, post fertilization is rapid cell division. It serves two purposes – (i) proliferative function to increase in cell number and (ii) gives rise to cellular diversity in the growing organism. While the former is taken care of by symmetric cell division, asymmetric cell division serves to generate cells of different lineages. Errors that are incorporated during this stage get permanently incorporated in the cell. Aberrations during cell division can lead to multiple problems which range from developmental anomalies, defective tissue organization and even uncontrolled cell proliferation leading to cancer. Cell division is under complex regulation from multiple players such as DNA compaction, regulation of cyclin/CDKs and check points. Another important determinant of cell division which ensures normal growth and development of an organism, and simultaneously also determines the future of daughter cells is the axis of cell division (Gönczy 2008; Morin and Bellaïche 2011; Gillies and Cabernard 2011).

Orientation of cell division establishes the content, position, and fate of daughter cells within tissues. First, this helps generate elongated cell sheets and define tissue shapes. Secondly, it determines cellular diversity. In order to achieve these two mutually non-exclusive functions, the orientation of the mitotic spindle needs to be controlled. Oscar Hertwig first recognized that spindle orientation and cell division orientation were instrumental in determining cell shape; he further discovered that cells divide along their long cell axis, an observation known as the ‘long axis rule’ (Hertwig 1984). Symmetric cell division refers to the equal partitioning of cellular constituents, giving rise to two identical daughter cells. On the other hand, asymmetric cell division gives rise to two non-identical daughter cells with unequal partitioning of cellular constituents.

Depending on their spatial orientation, shape, structure and function, cells may be broadly classified into two categories – polarized and non-polarized cells. During the early stages, post fertilization in mammals, rapid cell division is observed. These cells are pluripotent stem cells which undergo symmetric cell division to give rise to clonal population of cells. At some point during this clonal expansion, polarity is established using external and internal cues. Post this stage, the stem cells undergo both, symmetric division (for self-renewal) and asymmetric cell division (to give rise to differentiated cells of various lineages) (Fig 1a). The differentiated cells may be of polarized or non-polarized in nature (Gönczy 2008; Knoblich 2008; Morin and Bellaïche 2011; Gillies and Cabernard 2011;

Williams and Fuchs 2013; Lu and Johnston 2013). Non-polarized cells typically undergo symmetric cell division which serves to maintain cell number and homeostasis in an organism (Siller and Doe 2009). However, under the influence of external cues or improper spindle positioning, these might be able to divide with unequal partitioning of cellular constituents (i.e. asymmetric cell division) (Lancaster and Baum 2011) (Fig 1b). In case of polarized cells, the axis of division becomes a defining feature for the shape and characteristics of the daughter cells generated. Cells divide along the apico-basal axis to give rise to cells which belong to different niches. At an organismal level, this acts as one of the driving forces that guide the formation of the neural tube or alimentary canal from the polarized neuronal or epithelial cells. Polarized cells can also undergo division along the anterior-posterior axis and this division is governed by planar cell polarity (PCP pathway) (Simons and Mlodzik 2008; Devenport 2014; Morin and Bellaïche 2011) (Fig 1c). A battery of proteins and their gradients define the plane of the axis of division. These proteins determine the position and plane of divisional axis, which in turn gives rise to symmetric or asymmetric cell division. Both these divisions occur in case of polarized cells, based on external and internal cues. While the discussion on the effect of external cues is beyond the scope of the present discussion, the internal cues regulating the same are discussed briefly in this section.

Central to the machinery that regulates cell division axis and spindle positioning are partitioning family cell polarity regulator proteins. These determine the axis of division and gradient of proteins governing the same. Besides these, there are a host of cortically enriched proteins that assist in the correct division by translating the external and internal cues into cortical forces. These molecular interactions at the cell cortex in turn translate into the force necessary to hold the spindle apparatus in place and along the thus determined axis of division. The force required to stabilize the spindle apparatus in correct position and orientation, is generated by the interaction of astral microtubules with the cortical proteins and minus end directed motor protein, dynein. Dynein is recruited to the membrane by the help of an evolutionarily conserved ternary complex of proteins consisting of NuMa, LGN and G α -subunit of the trimeric G-protein complex (Lin-5/GPR-1/2/G α respectively in worm and Mud/Pins/G α in flies) (Du and Macara 2004; Siller and Doe 2009; Stevermann and Liakopoulos 2012; McNally 2013). Dynein serves as an anchor to maintain a connection with the depolymerizing microtubule, which then exerts the force necessary to position the spindle apparatus (Kotak et al. 2013) (Fig 1d). The details and intricacies of this process are

discussed in the course of this review in the context of different mitotic defects leading to disease conditions. Recent reports suggest that proteins participating in this regulation are mitotic motor protein KIF15 (Kinesin superfamily protein), Eg5 (also known as KIF11) (van Heesbeen et al. 2014), certain RanGTP and CLASP (Cytoplasmic linker associated protein 1) proteins (Bird et al. 2013). While CLASP1 is required for correct capture of astral microtubules, RanGTP and PLK1 ensure that the ternary complex is restricted to only the cell cortex and not the membrane area in vicinity of the metaphase plate. RanGTP has also been shown to promote astral microtubule elongation along the long axis of cells in *Xenopus* egg extracts (Zhang et al. 1999). Another aspect that is essential for the proper functioning of these cortical regulators is the integrity of the spindles formed during mitosis. If these fail to form stable structures during mitosis, no amount of cortical regulation can ensure accurate cell division.

Our understanding of the importance of post-translational modifications that regulate cortical proteins, spindle and astral microtubule polymerization and positioning is still premature. In this review, we discuss the emerging importance of ubiquitination in modulation of the molecular players affecting the orientation of cell division, leading to pathological abnormalities and disease conditions during their functional inactivation.

2. Microtubule assembly regulators

2.1 Ubiquitin E3 ligases

The covalent attachment of ubiquitin to specific protein substrates is achieved by the activity of E3 ubiquitin ligases. These ligases are divided into multiple classes defined by the structural element of their E3 functional activity. They are broadly classified into HECT (Homologous to E6-AP C-terminus)-family E3 ligase, and RING (Really Interesting New Gene). An extension of the RING family includes homeodomain (PHD) and leukemia associated protein (LAP) finger proteins, and members of the U-box family (a modified RING motif without the full complement of Zn²⁺-binding ligands) domain. While HECT E3s directly catalyse ubiquitination, RING and U-box E3s have been shown to facilitate this post-translational modification by primarily acting as scaffold proteins (Metzger et al. 2012). Both HECT and RING ligases transfer activated ubiquitin from a thioester to the ε-amino acid group of a lysine residue on the substrate. HECT ligases have an active site cysteine that forms an intermediate thioester bond with ubiquitin, on the other hand RING ligases interact with the ubiquitin conjugating enzymes to allow direct ubiquitin transfer from the E2 to substrate. Another family of ligases is the RBR family (Ring-Between-Ring) of proteins that function with a canonical RING domain and a catalytic cysteine residue usually restricted to HECT E3 ligases. Hence these ligases are also known as 'RING/HECT hybrid' enzymes (Wenzel et al. 2011; Stieglitz et al. 2012).

2.1.1 PARK2

Parkin (PARK2), an RBR ligase is widely implicated in the onset and progression of Parkinson's disease. Numerous reports suggest the dysregulation of the ubiquitin proteasome system (UPS) in the pathogenesis of Parkinson's disease (Hristova et al. 2009).

Recent studies show a more detailed and direct influence of PARK2 on cell division (Sun et al. 2013). Studies with pancreatic tumor samples from patients exhibit reduced expression levels of PARK2. Various mitotic defects were detected in these cases, vis-à-vis multiple spindle formation, chromosomal instability, spindle tilt along the axis of division and increase in the vertical distance between the spindle poles. These observations could be replicated in pancreatic cancer cell line, EPP85 with the depletion of PARK2. These

observations indicate an obvious involvement of PARK2, direct or indirect, in cell division (Sun et al. 2013).

It has been hypothesized that PARK2 modulates proteins critical for mitotic spindles via its regulation of the microtubule motor protein, Eg5 expression (Sun et al. 2013). Eg5 is a bipolar, plus-end-directed microtubule motor protein (Tanenbaum and Medema 2010). Depletion or inhibition of Eg5 activity prevents bipolar spindle formation and arrests cells in mitosis with unseparated centrosomes. Eg5 can crosslink microtubules into an antiparallel configuration and slide them apart, thus generating an outward-directed pushing force on centrosomes (Kapitein et al. 2005; Tanenbaum et al. 2009). Under normal cellular context, it has been shown that PARK2 mediates multi-monoubiquitination of Hsp70 which inactivates c-Jun NH2-terminal kinase, resulting in decreased phosphorylation of c-Jun. This leads to reduced c-Jun binding to the activator protein 1 site present in the Eg5 promoter and downregulation of Eg5 gene transcription (Liu et al. 2008) (Fig 2). Hence, under conditions when PARK2 ligase activity is repressed, Eg5 activity is indirectly elevated resulting in aberrant mitotic apparatus.

However, the role of Eg5 in cell division is contentious. Eg5 has been demonstrated to participate in prophase centrosome separation in mammalian cell systems (Blangy et al. 1995; Sawin and Mitchison, 1995; Whitehead et al. 1996), while in *Drosophila* embryos, its homolog does not have a similar role in prophase (Sharp et al. 1999). Moreover, even in mammalian systems, prophase centrosomes have been suggested to move independently of each other without the involvement of Eg5 (Waters et al. 1993). Hence, the importance/function of Eg5 in bipolar spindle assembly is far from clear.

Thus, though there are evidences for the regulation of spindle tilt by PARK2, the exact mechanism still needs to be clearly understood. It is plausible that PARK2 regulates spindle tilt and pole formation in an Eg5 independent manner.

2.1.2 BRCA1/BARD1

Germline mutations in Breast cancer-associated gene 1 (BRCA1) have been associated with familial breast and ovarian cancers. Further, downregulation of BRCA1 has been reported in sporadic breast and ovarian cancers. BRCA1 undergoes heterodimerisation with BRCA1-associated RING domain protein, BARD1 (Wu et al. 2006). The RING

domains of BRCA1 and BARD1 form a dimerization interface and the BRCA1/BARD1 heterodimer functions as an ubiquitin ligase (Parvin 2009). This has been shown to participate in cell proliferation and chromosome stability.

Homozygous *Brca1* knockout mice die *in utero* between 10 and 13 days of gestation (E10-E13). These mice have abnormalities in the neural tube, with 40% of the embryos showing varying degrees of spina bifida and anencephaly. Further, the neuroepithelium in *Brca1*-deficient embryos also seem to be disorganized (Parvin 2009). At the cellular level, BRCA1 and BARD1 localize to the centrosome during the cell cycle (Sankaran et al. 2005). Embryonic fibroblasts from *Brca1* knockout mice show centrosome amplification and genome instability (Kais et al. 2012). BRCA1 is known to bind to γ -tubulin, a major component of centrosomes. Studies in *Xenopus* egg extracts and HeLa cells show that this BRCA1/BARD1 complex then mono-ubiquitinates γ -tubulin on Lysine 48 (K48) and K344 (Joukov et al. 2006). The BRCA1/BARD1 ubiquitin ligase activity regulates centrosome number and function. Further, BRCA1/ BARD1-dependent ubiquitination activity modulates the microtubule nucleation and centrosomal hypertrophy (Sankaran et al. 2005). Cells with depleted BRCA1/BARD1 show suppressed aster formation in interphase (Tarapore et al. 2012). BRCA1/BARD1 is also required for mitotic spindle pole assembly and for the accumulation of TPX2 (targeting protein for Xklp2), a major spindle organizer and a downstream target of Ran-GTP, on spindle poles. This function is centrosome independent, operates downstream of Ran GTPase, and depends upon BRCA1/BARD1 E3 ubiquitin ligase activity (Daniels et al. 2004) (Fig 3).

As indicated before, the microtubule aster polymerization and spindle pole assembly is an important player that regulates the positioning of the spindle apparatus in the center of the cell, along the planar axis of division during in case of polarized cells. These observations about BRCA1/BARD1 can thus be extrapolated to explain the increased propensity for breast cancer in individuals who harbor mutations in these genes.

BRCA1/BARD1 is known to localize to centrosomes during metaphase where this ubiquitinates and degrades Aurora B (Ryser et al. 2009). The same study has elucidated that various cancer cells express RING-deficient isoforms of BARD1, called BARD1 β . This isoform can have BRCA1-independent functions (Ryser et al. 2009). It has been shown that BARD1 β localizes to the midbody at telophase and cytokinesis, where it associates with and stabilizes Aurora B, a protein crucial for midbody formation and abscission (Delaval et al.

2004; Ryser et al. 2009). Thus, the elevated levels of BARD1 β and the imbalance between the BARD1/BARD1 β levels in cancer cells could partially contribute to their increased rate of cell division.

2.1.3 VHL

von Hippel-Lindau disease, an inherited cancer syndrome is associated with an E3-ligase, VHL (von Hippel-Lindau tumor suppressor) in which patients are predisposed to develop various vascular tumors and clear cell renal cell carcinomas (ccRCC) highlighting distinct roles for VHL in tissue morphogenesis and tumorigenesis (Kaelin 2007). It is the substrate recognition component of the cullin-RING ubiquitin ligase complex (CRL) that includes cullin-2, Rbx 1, and elongins B and C (Deshaies and Joazeiro 2009; Kaelin 2008). VHL is best characterized for its role as a key component of an E3 ubiquitin ligase that targets hypoxia-inducible factor (HIF) for degradation (Kaelin 2008; Jaakkola et al. 2001). The homozygous knockout of *Vhl* leads to embryonic lethality at E14.5 to E18.5. Heterozygous knockout mice however, appear normal in their development, growth and reproductivity (Zhang et al. 2012).

VHL is known to play a role in spindle orientation as its knockdown in MEFs and HeLa causes spindle misorientation by eliminating astral microtubules. The defects observed by VHL downregulation bear striking resemblance to that of APC (Adenomatous polyposis coli) inactivation which includes compromised spindles and misaligned chromosomes (Thomma et al. 2009). One of the possible reasons might be that VHL positively regulates MAD2 (mitotic arrest deficient 2) levels in cells (Thoma et al. 2009; Liu et al. 2011). MAD2 is a member of mitosis checkpoint complex at the kinetochore, where it interacts with other proteins like Bub1 (Budding uninhibited by benzimidazoles 1 homolog). MAD2 reduction is seen to cause aneuploidy in cells, an observation widely prevalent in VHL-defective cells as well. This is consistent with the known contribution of VHL to microtubule stability and cell cycle regulation. Further, VHL may be required for the degradation of incorrectly folded tubulin, suggesting that VHL could also contribute to MT dynamics (Delgehyr et al. 2009).

2.1.4 MGRN1

Mahogunin ring finger-1 (MGRN1) was identified by positional cloning of mahoganoid (*md*) (Miller et al, 1997), a mutation characterized by darkening of coat color in mice. Mutant mice show changes in the levels of expression and activity of mitochondrial proteins in the brain which precedes the development of spongiform neurodegeneration with many features of prion diseases (Cota et al. 2006; Jiao et al. 2009). Although the mechanisms leading to spongiform neurodegeneration are not fully understood, data shows that some of the disease causing abnormal isoforms of PrP (transmembrane isoform, ^{Ctm}PrP and cytoplasmic isoform, cyPrP), can engage in atypical interactions with MGRN1 (He et al. 2003; Kim et al. 2007; Chakrabarti and Hegde. 2009). Independent studies indicate that while nearly 50% of *Mgrn1*-null mutants in mice develop late-onset spongiform neurodegeneration, rest exhibit severe developmental defects (like congenital heart defects, abnormal craniofacial patterning and mispatterning of the left-right (LR) body axis) and increased mortality by weaning age, thus suggesting multiple roles for MGRN1. Additionally, about 1% of animals also suffer from *situs inversus* (complete reversal of the left and right body axes) (Cota et al. 2006; Jiao et al. 2009).

Recent study from our laboratory shows that α -tubulin is a substrate for MGRN1 (Srivastava and Chakrabarti 2014). It is shown to polyubiquitinate α -tubulin and promotes its polymerization. This report further shows that in cell culture systems (HeLa and MEFs), mitotic cells which over-express functionally inactive forms of MGRN1 exhibit multiple spindle defects ranging from spindle tilt and shortening of aster microtubules. It is possible that the poorly formed spindles cause an inherent instability in the spindles. These malformed spindles may not engage in robust interactions with cortical proteins, leading to the observed defects. It would be worth exploring if MGRN1 regulates other players to affect the spindle positioning. Unpublished data from our laboratory shows that MGRN1 modulates the cortical enrichment of the proteins involved in spindle positioning (Fig 4). These studies give us an attractive handle to study and dissect the activity of MGRN1 in cell culture systems and explore the cause of the pleiotropic effects observed in its absence during embryonic development. The modulatory effect of MGRN1 on the microtubules may not, however, directly affect spongiform neurodegeneration.

2.1.5 SIAH1

Besides the above discussed E3-ligases there are several other ubiquitinating enzymes that regulate spindle positioning and thus might be potential players in cancer. The human homologue of the *Drosophila seven in absentia* (Sina) (SIAH1 and SIAH 2), have been implicated in ubiquitin-mediated proteolysis of different target proteins and are known to regulate HIF α protein stability. SIAH1 and SIAH2 bind Propyl Hydroxylases (PHD1 and PHD3) and target them for proteasomal degradation (Nakayama et al. 2004). Double knockout mice for *Siah1* and *Siah2* are not viable, but single knockouts are able to live. Growth in *Siah1* knock-out mice is severely retarded and along with poor bone formation (House et al. 2009). Also, *Siah1* null mice have poor viability and display a meiotic block at metaphase I that impairs spermatogenesis and causes sterility in male mice (Dickens et al. 2002).

Murine *Siah-1* is a p53-induced gene; its human homolog, *SIAH-1* is activated during tumour suppression and p53 induced apoptosis. When overexpressed, it has been shown to inhibit cell growth by causing defects in mitosis and increasing apoptosis in breast cancer cell line, MCF-7. Mitotic defects include anomalies of the mitotic spindle and abnormal chromosomal segregation, multinucleate giant cells along with failure of cytokinesis. Established targets of SIAH1 include α -tubulin and Kid (kinesin like DNA binding protein, also known as KIF22), identified by yeast two hybrid assays (Germani et al. 2000). While the significance of interaction with α -tubulin is mostly speculative, it has been shown to ubiquitinate and degrade Kid. Kid has been proposed as a linkage regulator of chromosome movement along microtubules during mitosis. Reports showed that the *Xenopus* homologue of Kid (Xkid) is the motor protein that provides the necessary force to push chromosome arms toward the equator of spindle. Its degradation is necessary for anaphase chromosome movement. These observations provide a plausible explanation for the mitotic anomalies observed in the absence of SIAH1 (Antonio et al. 2000; Funabiki and Murray, 2000; Germani et al. 2000).

Interestingly, SIAH1 is also shown to polyubiquitinate microtubule plus-end tracking proteins (+TIP) binding protein EB3 (End Binding protein 3), and mediate its degradation through the ubiquitin-proteasome system. EB3 has been shown to facilitate cell cycle progression at prometaphase, and is downregulated during the transition to G1 phase in cell

culture based studies (Ban et al. 2009). Thus, the interaction between SIAH1 and EB3 could be used as an alternative explanation for the observed mitotic defects and spindle anomalies.

2.1.6 SMURF2

Another interesting ligase is SMURF2 (Smad specific ubiquitin regulatory factor 2) which is a HECT E3 ligase, originally characterized as a negative regulator of the TGF- β signaling pathway by targeting receptors, signaling intermediates, and other pathway-specific transcription factors for degradation (Tang et al. 2011). *Smurf2*^{-/-} mice (>99%) do not exhibit any pronounced developmental defects during embryogenesis and they are phenotypically indistinguishable from their wildtype or heterozygous littermates at weaning (Lin et al. 2000). Surprisingly mice mutant for both *Smurf1* and *Smurf2* display planar cell polarity (PCP) defects in the cochlea and abnormalities in convergence and extension movements (CE), which include a failure to close the neural tube. Further, it has also been illustrated that SMURFs are involved in non-canonical Wnt signaling pathway via ubiquitin-mediated degradation of the core PCP protein Prickle1 (Narimatsu et al. 2009).

SMURF2 exhibits a dynamic localization pattern throughout mitosis, moving from centrosomes in late prophase and metaphase, to the mitotic midzone in anaphase, and ultimately to the midbody in telophase. Study shows that SMURF2 participates in posttranslational control of MAD2 (mitotic arrest deficient 2), stabilizing the same. SMURF2 depletion results in enhanced polyubiquitination and degradation of MAD2. MAD2 mislocalizes in SMURF2-depleted cells, suggesting that SMURF2 regulates the localization and stability of MAD2, thus regulating spindle check point. In addition to its role as a regulator of the spindle checkpoint, the localization of SMURF2 to other critical mitotic structures implies its participation in other aspects of mitotic control. Further studies would be required to establish this hypothesis (Osmundson, 2004).

2.1.7 TRIM E3 ligases

A family of E3 ligases worth mentioning is TRIM family (The conserved arrangement of a RING-finger domain, B-box, and coiled-coil domains). Several members of this family of ligases have been implicated in disease conditions namely, MID1 (Midline-1), TRIM5 α

(Tripartite motif protein 5- α) and Xnf7 (*Xenopus* nuclear factor 7). While TRIM5 mediates resistance to the human virus in rhesus monkey cells (Stremlau et al. 2004), MID1 is implicated in developmental disorder, Opitz Syndrome, an inborn error of cholesterol synthesis (Cox et al. 2000).

Xnf7 (*Xenopus* nuclear factor 7), discovered from *Xenopus* egg extracts as an inhibitor of the anaphase promoting complex (APC), is a maternally expressed nuclear protein that is retained in the cytoplasm from oocyte maturation until the midblastula transition (MBT) and is shown to be involved in dorsal/ventral patterning (El- Hodiri, 1997b). Xnf7 has not yet been implicated in disease condition. Although both MID1 and Xnf7 have E3 ligase activities, their roles in governing spindle dynamics do not involve this enzymatic activity. These proteins bind to microtubules and drive their bundling, thus acting as key players in the regulation of spindle dynamics (Maresca et al. 2005).

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2.2 Other UPS associated proteins regulating spindle dynamics

2.2.1 HUNTINGTIN (HTT)

Huntingtin (HTT), implicated as the foremost player in the onset and progression of the severely debilitating neurodegenerative disease, Huntington's disease (HD), is also suggested to modulate spindle assembly and orientation. HD is a neurodegenerative disorder characterized by severe psychiatric, cognitive, and motor defects and selective neuronal death in the brain, caused by huntingtin (*HTT*) gene. Although the mechanisms leading to disease are not completely known, increasing evidences indicate that in addition to the gain of new toxic properties, loss of wild-type HTT function also adds to pathogenesis (Cattaneo et al. 2005). Various studies in cell models and with patient samples have reported that the UPS is impaired in HD, which could be the underlying cause of the neurotoxicity.

Htt is widely expressed in the early embryonic stages in mice where it plays an important role in several processes including cell differentiation and neuronal survival. Inactivation of the mouse gene results in developmental retardation and embryonic lethality at E7.5 (Nasir et al. 1995; Zeitlin et al. 1995). Studies have now conclusively shown that HTT is involved in neurogenesis and brain development (Godin et al. 2010).

Reports suggest that HTT is ubiquitinated and it also interacts with a ubiquitin conjugating enzyme E2 (Kalchman et al. 1996). The ubiquitinated species detected may be parts of either processed or partially degraded forms of HTT. Studies in SHSY5Y cells show that stable expression of long polyglutamine tracts lead to cell cycle arrest and inhibition of proteasomal degradation pathway. The impaired proteasomal activity does not elevate protein aggregation under basal conditions, however it severely compromises the ability of the proteasome to respond to stress leading to increased stress-induced protein aggregation. This is further accompanied by altered expression of the 20s proteasomal subunits (Ding et al. 2002).

Recent studies have shed a lot of light on the function of HTT in the cell. Wild type HTT is shown to interact with microtubules, the dynein/dynactin complex and kinesin to regulate the microtubule-dependent transport of organelles in neurons (Caviston et al. 2007; McGuire et al. 2006). HTT is found in high levels in dividing cells where it associates with the centrosomal region and microtubules (Hoffner et al. 2002). Interestingly, HTT has been shown to be required in murine neuronal progenitors for appropriate spindle orientation and

for cell fate determination (Godin et al. 2010). Also, it has been demonstrated that mutant forms of HTT disturb the cortical localization of proteins like dynein, NuMa (Molina-Calavita M et al. 2014). A study in mammary cells throws light on the mechanistic cause of the mitotic abnormalities observed earlier. This study shows that HTT is not only important for bipolar spindle assembly but also for the cortical localization of the dynein/dynactin/NUMA/LGN complex. Thus HTT may be a connecting molecular link between the dynein/dynactin and NUMA/LGN pathways ensuring the transport of the dynein/dynactin/ NUMA/LGN complex from the spindle poles to the cell cortex. HTT is known to interact with kinesin 1 (KIF5) through the Huntingtin associated protein 1 (HAP1) to promote microtubule-based anterograde vesicular transport in neurons (Colin et al. 2008; McGuire et al. 2006). During mitosis, HTT is targeted to the spindle poles due to its interaction with dynein and there it promotes the accumulation of NUMA and LGN. It has been shown that KIF5 is a molecular plus-end motor, which in association with HTT, regulates the trafficking of the dynein/dynactin/ NUMA/LGN complex along the astral microtubules to the cell cortex during mitosis. Once at the cell cortex, the dynein/dynactin/NUMA/LGN complex generates pulling forces on astral microtubules for mitotic spindle positioning in the center of the cell (Elias et al. 2014).

Yet unclear, it will be interesting to closely study the role of HTT and see if there is any direct or indirect connection between its influence on the regulation of UPS cells, the subsequently reported cell cycle inhibition and the regulation of the spindle apparatus.

2.2.2 Deubiquitinases

Besides the ubiquitin ligases, the deubiquitinating enzyme, ubiquitin C-terminal Hydrolase L1 (UCH L1) also plays a significant role in modulating microtubule dynamics. UCH L1 is a cysteine hydrolase that contains a typical active-site triad of cysteine, histidine, and aspartic acid and catalyzes hydrolysis of C-terminal esters and amides of ubiquitin. This enzyme is expressed in abundance in brain and reproductive tissues (Setsuie and Wada, 2007). Reports indicate that mutations in the UCH L1 gene have been involved with Parkinson's and Alzheimer's diseases (Betarbet et al. 2005). Recent studies indicate that UCH L1 is multi-functional protein: besides being a deubiquitinating enzyme, UCH L1 dimer has an ubiquitin ligase activity *in vitro*. It has been implicated in mitosis by studies in cell

culture systems, where it binds to tubulin and regulates microtubule dynamics by ubiquitination (Bheda et al. 2010).

The deubiquitinating enzyme cylindromatosis (CYLD), specifically removes lysine 63 (K63)-linked polyubiquitin chains and is a regulator of microtubule dynamics (Gao et al. 2008; Harhaj and Dixit 2011). It stabilizes astral microtubules and stimulates the formation of the Dvl (Disheveled)-NuMA-dynein/dynactin complex at the cell cortex in Hela cells and mice models. The deubiquitination of Dvl enhances its interaction with nuclear mitotic apparatus, stimulating its cortical localization in association with the dynein/dynactin motor complex. Thus generates pulling forces on astral microtubules (Yang et al. 2014). These studies suggest a role for CLYD in tumor progression as it is well known that the tumor suppressor protein is mutated in familial cylindromatosis and multiple familial trichoepithelioma, genetic conditions associated with the development of skin-appendage tumors (Massoumi 2011).

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3. Conclusion

From the few E3 ligases discussed above, it is evident that these proteins play an important role in cell division. This review only highlights the importance of the ubiquitin E3 ligases that directly interact with or alter mitotic spindle generation, positioning and orientation (Fig 5). It is essential to point out that there is an elaborate list of other E3 ligases, like APC and Skp, Cullin, F-box containing complex (or SCF complex) which also affect cell cycle progression and mitosis, either directly by modulating the cell cycle check points or indirectly, by altering the functions of other MT interacting proteins. Surprisingly, while all the ligases discussed in this review are crucial for successful mitosis, all of them except BRCA1 and VHL are non-essential – in other words their loss of function does not lead to embryonic lethality. One probable explanation could be a phenomenon similar to what is observed in *Drosophila* during development -- “telophase rescue”. It redistributes fate determinants in accordance with spindle orientation just before cytokinesis in the majority of mutant neuroblasts, irrespective of the AB polarity axis. Mutations in *mud* (homolog of NuMa present in *Drosophila*) are only able to cause defects in spindle orientations but not affect the polarity axis during division. The mechanism of “telophase rescue” are not entirely clear (Schober et al. 1999; Wodarz et al. 1999; Peng et al. 2000; Neumüller and Knoblich, 2009; Morin and Bellaïche, 2011). It is plausible to hypothesize that similar salvage mechanisms exist in mammalian systems as well, thus ensuring correct division during development. Alternatively, it also raises the possibility of functional redundancy between the isoforms of the proteins and amongst different types of proteins as well. Though it may not have been studied in depth in the context of the ubiquitin E3 ligases, this concept is not new as there exists examples of functionally redundant proteins in the genome (Kafri et al. 2006). It has been shown that responsive backup circuits may function as devices for filtering non-genetic noise from transcriptional pathways, thus rendering an added layer of protection to the organism against stress and mutations (Massoumi 2011). More extensive genome wide studies are required to prove that similar backup circuits exist with respect to E3 ligases as they may seem functionally irreplaceable at the cellular levels, yet they are non-essential at the organismal level during development.

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Draft

Figure Legends

Figure 1: Types of cell division in the various cell types in the body. (a) Schematic diagram showing the relevance of symmetric/asymmetric division in stem cells. (b) This shows one of the possible ways by which external cues could cause erroneous spindle positioning in the cell leading to asymmetric division in non-polarized cells. Internal cues, like altered distribution of the cortical regulators could also give rise to a similar situation. (c) Symmetric and asymmetric division in case of polarized cells is shown. While the former is seen along the anterior –posterior axis, division along the apico-basal axis leads to asymmetric cell division. (d) An overview of different the cortical players important for spindle orientation. LGN acts as an adaptor protein which holds NuMa and $G\alpha_i$. This ternary complex recruits dynein at the cell cortex by the help of motor proteins. NuMa helps establish a cortical zone enriched in dynein by binding to it. Dynein then binds the astral microtubules and regulates spindle positioning in cells. Polo Kinase 1 present on spindles serves to negatively regulate the cortical dynein localization while the RanGTP gradient present on the chromosomes negatively regulates NuMa/LGN distribution in the lateral cell cortex. It is the cooperative balance of these two gradients that positions the cortical proteins in the right place, thus ensuring correct spindle positioning. Further, players like CLASP1 (*not shown in the figure*) belonging to a family of Microtubule Associated Proteins (MAPs), are involved in the correct capture of the astral microtubules and their attachment at the cell cortex.

Figure 2: PARK2 and spindle defects. Schematic of the possible mode of action of PARK2 by which it affects spindles and causes spindle multipolarity.

Figure 3: BRCA1: centrosomal and spindle defects. Summary of the mode of action of BRCA1 by which it affects spindles and causes genomic instability.

Figure 4: MGRN1 and spindle tilt. An overview of the effect of MGRN1 on spindles and its plausible mode of action.

Figure 5: A summary of the different target proteins/ structures regulated by ubiquitination in the cortex of the cell during mitosis. Ligases like MGRN1, VHL, Xnf7 and SIAH1 regulate microtubule assembly or polymerization, while BRCA1/BARD1 regulates the centrosomal apparatus. PARK2 and HTT affect microtubule motors, thus in turn regulating the correct localization of the cortical effectors of mitosis.

Types of cell division in the various cell types in the body.
229x165mm (300 x 300 DPI)

Figure 2

Figure 3

BRCA1: centrosomal and spindle defects.
143x76mm (300 x 300 DPI)

Figure 4

Figure 5

A summary of the different target proteins/ structures regulated by ubiquitination in the cortex of the cell during mitosis.
110x76mm (300 x 300 DPI)