Chapter 10
Molecular Links Between Centrosome Duplication and Other Cell Cycle-Associated Events

Kenji Fukasawa

Abstract The centrosome duplicates once in each cell cycle, and the duplication proceeds in coordination with other cell cycle events. Thus, centrosome duplication must crosstalk with other cell cycle events, including the growth signaling and DNA replication. Recent studies have identified several pathways that link the receptor tyrosine kinases (RTKs) activation and initiation of centrosome duplication. The molecular mechanisms of how those pathways link the RTK activation and initiation of centrosome duplication will be discussed in the first part of this chapter. Because centrosome duplication occurs at the time of S-phase entry, there is a mechanism that couples the initiation of centrosome duplication and DNA replication, and cyclin-dependent kinase 2 (CDK2)-cyclin E kinase complex is known to play a key role. In the second part of this chapter, through focusing of the p53-p21 pathway, the regulatory mechanisms underlying the coupling of initiation of centrosome duplication and DNA replication, and how loss of p53 leads to overduplication of centrosomes (centrosome amplification) will be discussed.

K. Fukasawa (✉)
Molecular Oncology Program, H. Lee Moffitt Cancer Center & Research Institute,
SRB-2, 12902 Magnolia Drive, Tampa, FL 33612-9416, USA
e-mail: kenji.fukasawa@moffitt.org

10.1 Link Between Activation of Growth Factor Receptors and Initiation of Centrosome Duplication: The Roles of the Rho-ROCK II Pathway and STAT Transcriptional Factors

When RTKs are activated by growth factor binding, they transmit the growth signals to a number of different pathways, and cells commence cell cycling processes. Because centrosome duplication is a cell cycle-dependent event, it is reasonable to predict that the activated RTKs also signal to initiation of centrosome duplication. It has been known that there is a close link between the activation of RTKs and initiation of centrosome duplication. For instance, in certain cell types, addition of epidermal growth factor (EGF) can rather rapidly induce physical separation of paired centrioles, which is an initial event of centrosome duplication (Sherline and Mascardo 1982). In the experimental system using Chinese hamster ovary cells that are cell cycle-arrested by exposure to DNA synthesis inhibitors, centrosomes continue to duplicate, resulting in generation of ≥3 centrosomes (centrosome amplification), but when serum is depleted from the media, centrosomes are no longer able to undergo duplication. Moreover, centrosomes resume duplication and reduplication in those arrested cells upon addition of serum or EGFs to the media (Balczon et al. 1995). It has also been known that oncogenic (constitutive) activation of RTKs such as the Met receptor leads to centrosome overduplication and amplification (Kanai et al. 2010; Nam et al. 2010; Fukasawa 2011).

What is the molecular pathway(s) that link the activation of RTKs occurring at cell membrane to the initiation of centrosome duplication occurring near the nuclear membrane? This question has recently been answered at least in part by the identification of ROCK II kinase as a key positive regulator of centrosome duplication (Ma et al. 2006) (Fig. 10.1). ROCK II is one of two members of the ROCK Ser/Thr kinase family. ROCK II is primed for activation by binding of GTP-bound Rho small GTPase (Rho-GTP): Rho binding disrupts the interaction between the kinase domain and autoinhibitory domain of ROCK II, freeing the kinase domain (Leung et al. 1996; Matsui et al. 1996). Rho cycles between an active GTP-bound state and inactive GDP-bound state, and many RTKs, when activated by the ligand binding, promote the exchange for Rho-bound GDP to GTP via activating the Rho guanine nucleotide exchange factors (Rho-GEFs) (Etienne-Manneville and Hall 2002). ROCK II was found to localize to centrosomes, and ectopic expression of the ROCK II mutant that lacks the negative regulatory domain (hence its activity is independent from Rho-binding) promotes initiation of centrosome duplication in a kinase activity and centrosome localization-dependent manners. Moreover, depletion of ROCK II results in significantly delayed initiation of centrosome duplication, indicating that ROCK II plays a critical role in the timely initiation of centrosome duplication. Of note, although the initiation of centrosome duplication is delayed in the ROCK II-depleted cell, they eventually duplicate because of the functional replacement by ROCK I, another member of the ROCK family, that shares ∼65 % overall identity
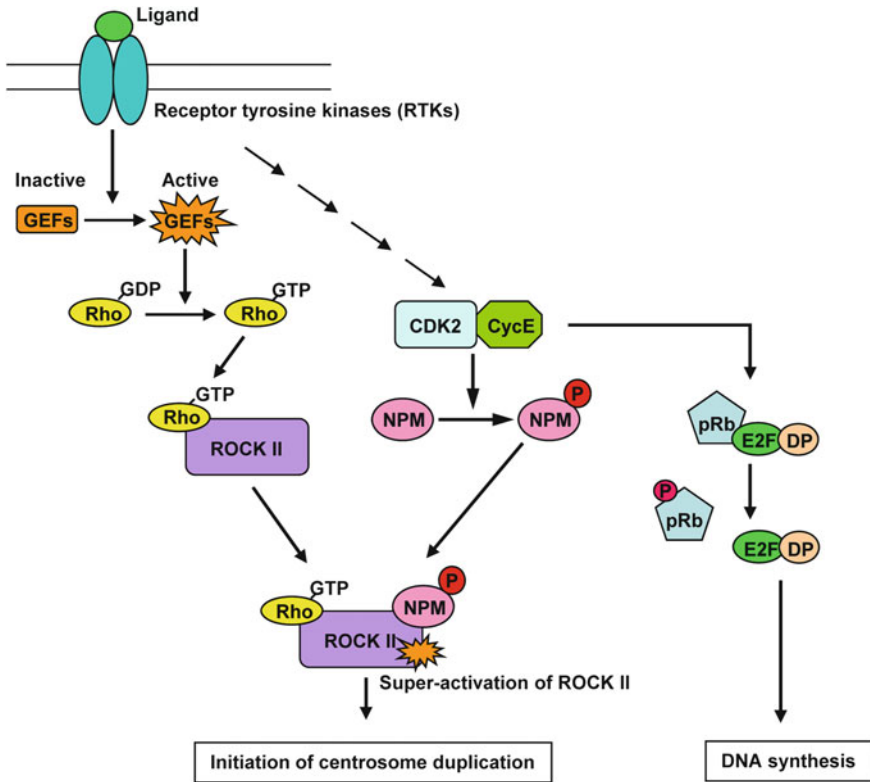
Fig. 10.1 The Rho-ROCK II and CDK2/cyclin E-NPM pathways link the RTK activation and initiation of centrosome duplication. The RTKs activated by the binding of growth factors activates Rho-GEFs, which in turn promotes exchange of Rho-GDP to Rho-GTP. Rho-GTP is then recruited to centrosomes, and binds to ROCK II at centrosomes. In late G1 phase, CDK2-cyclin E is activated, and phosphorylates NPM/B23 likely at centrosomes. NPM/B23 acquires a high binding affinity to ROCK II upon phosphorylation by CDK2-cyclin E, and binds to and superactivates ROCK II. The super activated ROCK II then triggers initiation of centrosome duplication. At the same time, CDK2-cyclin E targets proteins, including pRB which inhibits E2F transcriptional factor through direct binding. Upon phosphorylation by CDK2-cyclin E, pRB dissociates from E2F, resulting in activation of E2F and consequentially initiation of DNA replication

with ROCK II (Nakagawa et al. 1996). ROCK I also localize to centrosomes, and is implicated in proper positioning of centrosomes (Chevrier et al. 2002). ROCK I is dispensable for initiation of centrosome duplication as long as ROCK II is present. However, in the absence of ROCK II, ROCK I comes into replace the ROCK II function to promote centrosome duplication, but not as efficiently as ROCK II, resulting in the delay in the initiation of centrosome duplication. The reason behind the inefficient triggering of centrosome duplication by ROCK I is that unlike ROCK II, ROCK I cannot be super activated by nucleophosmin binding (discussed in details below) (Ma et al. 2006).

ROCK II is present at centrosomes throughout the cell cycle, and activated Rho (Rho-GTP) proteins are found at centrosomes much more than the inactive Rho (Rho-GDP) proteins (Kanai et al. 2010). Thus, Rho is likely recruited to centrosomes as Rho-GTP (after activation by Rho-GEFs), and binds to ROCK II at centrosomes. There are three major Rho isoforms, RhoA, B and C, and they share 85 % sequence identity (Etienne-Manneville and Hall 2002), yet each isoform is known to function in the specific cellular events (Wheeler and Ridley 2004). Although all isoforms are capable of binding and activating ROCK II in vitro, RhoA and RhoC, but not RhoB, are involved in the regulation of centrosome duplication. For instance, ectopic expression of constitutively active forms of RhoA (RhoA-V14) as well as RhoC (RhoC-V14) leads to promotion of centrosome duplication, while expression of RhoB-V14 has no effect on centrosome duplication (Kanai et al. 2010). The inability of RhoB to function in the regulation of centrosome duplication appears to be in part by its inability to localize to centrosomes. Although the primary target of RhoA and RhoC appears to be ROCK II for the regulation of centrosome duplication, both RhoA and RhoC are required for centrosome duplication. For instance, depletion of either RhoA or RhoC alone results in inhibition of centrosome duplication. Since expression of excess RhoA in the RhoC-depleted cells as well as expression of excess RhoC in the RhoA-depleted cells allow centrosome duplication, it is likely that RhoA and RhoC comprise the total amount of Rho proteins necessary for activating ROCK II (especially those present at centrosome) to promote centrosome duplication (Kanai et al. 2010). However, it remains as a possibility that RhoA and RhoC may also activate distinct targets in addition to ROCK II for promoting centrosome duplication.

Because activated RTKs signals to a number of pathways, the pathways other than the Rho-ROCK II pathway may also be involved in the promotion of centrosome duplication. For instance, activation of many RTKs leads to upregulation of STAT (signal transducer and activator) transcriptional factors. The activity of STAT3 has been shown to be essential for centrosome duplication by inducing the expression of some key centrosomal proteins such as PCM-1 and γ-tubulin (Metge et al. 2004). STAT3 induces expression of those proteins not by direct upregulation of the transcription of the respective genes, but does so indirectly likely by upregulating other transcriptional factor(s). STAT5, another member of the STAT family, has also been shown to promote centrosome duplication via inducing expression of Aurora-A (also known as STK15 and BTAK) (Hung et al. 2008), which is a positive regulator of centrosome duplication (Zhou et al. 1998). This study shows that the ligand-activated EGF receptor is translocated into the nucleus, and recruited to the AT-rich sequence sites of the Aurora-A promoter through interacting with STAT5.

In sum, RTKs activated by growth factor binding transmit the signal to centrosomes to duplicate through activation of the Rho-ROCK II pathway and transcriptional induction of the key centrosomal proteins and positive regulatory protein(s) essential for centrosome duplication. It should be noted here that other downstream pathways of RTKs may also function to link the RTK activation and

initiation of centrosome duplication. For instance, the Ras-MAPK pathway and Akt (protein kinase B) pathway are both implicated in the regulation of centrosome duplication (Fukasawa and Vande Woude 1997; Zeng et al. 2010; Nam et al. 2010). Further studies should reveal the underlying mechanisms of the regulation of centrosome duplication by these pathways.

Overexpression and oncogenic mutation of RTKs are highly common in various types of cancers. It had been known that chromosomes become destabilized in cells transformed by oncogenically activated RTKs, but such a phenomenon had been belittled as an indirect consequence of the continuous firing of growth signals. However, the recent findings described above indicate that oncogenic activation of RTKs influences chromosome stability more directly than previously being thought via induction of centrosome amplification through upregulation of the Rho-ROCK II pathway and possibly other pathways as well as STAT-dependent transcription. Considering that chromosome instability plays a critical role in tumor progression, and centrosome amplification is one of the major causes of chromosome instability in cancer cells, induction of centrosome amplification and consequential destabilization of chromosomes should be appreciated as one of the key oncogenic activities of many RTKs.

10.2 Link Between Activation of Initiation of Centrosome Duplication and DNA Replication: The Roles of CDK2-Cyclin E and p53

As described in other chapters, CDK2-cyclin E plays a key role in the initiation of centrosome duplication (Hinchcliffe et al. 1999; Lacey et al. 1999; Tarapore et al. 2002). In normal cells, initiation of centrosome duplication occurs at the time of S-phase entry. Because CDK2-cyclin E is also a key triggering factor for DNA replication (Dulic et al. 1992; Koff et al. 1992), the coupling of initiation of centrosome duplication and DNA replication is at least in part achieved by the late G1-specific activation of CDK2-cyclin E resulting from the temporal increase of cyclin E expression. One of the targets of CDK2-cyclin E is nucleophosmin (NPM/B23). NPM/B23 is involved in the regulation of centrosome duplication both positively and negatively (Okuda et al. 2000; Grisendi et al. 2005; Ma et al. 2006), and CDK2-cyclin E-mediated phosphorylation of NPM/B23 on Thr199 residue simultaneously switches off the negative regulatory function and switches on the positive regulatory function. By the mediation of the Thr199-phosphorylated NPM/B23, the CDK2-cyclin E pathway and Rho-ROCK II pathway come together to trigger initiation of centrosome duplication (Fig. 10.1). ROCK II is not fully activated by the Rho binding: Rho binding results in only 1.5-fold increase in the kinase activity (Amano et al. 1996). NPM/B23 physically interacts with Rho-bound ROCK II (the NPM/B23-binding region of ROCK II is located near the kinase domain, and is masked by the negative regulatory domain in the nascent

form of ROCK II, and thus NPM/B23 cannot bind to Rho-unbound ROCK II), and ROCK II is super activated (5–10-fold higher than unbound ROCK II) by the NPM/B23-binding (Ma et al. 2006). Although unphosphorylated NPM/B23 can bind to ROCK II, NPM/B23 acquires a significantly higher binding affinity to ROCK II upon Thr199 phosphorylation. Under a physiological condition where the protein concentrations are limited, especially the Rho-bound ROCK II at centrosomes, phosphorylation-dependent upregulation of the ROCK II-NPM/B23 interaction becomes essential. Indeed, most (if not all) of the ROCK II-bound NPM/B23 in cells are Thr199-phosphorylated. The superactivation of ROCK II by NPM/B23 binding is critical for the timely initiation of centrosome duplication, and is the primary downstream event of CDK2-cyclin E for the initiation of centrosome duplication. For instance, downregulation of the CDK2 activity either by expression of the dominant negative CDK2 or by depletion of cyclin E and cyclin A results in complete inhibition of centrosome duplication (Hanashiro et al. 2008), but introduction of the Rho-independent constitutively active ROCK II mutant can override the inhibition of centrosome duplication by inactivation of CDK2 in a NPM/B23 binding-dependent manner (Hanashiro et al. 2011). To sum up, in late G1, NPM/B23 acquires a high binding affinity to ROCK II by CDK2-cyclin E mediated phosphorylation, and binds to and superactivates the Rho-bound ROCK II, which in turn rapidly acts on the centrosomal target(s) to initiate centrosome duplication. At the same time, CDK2-cyclin E targets proteins like Rb to initiate DNA replication, and thus initiation of centrosome duplication and DNA replication occurs in a coordinated manner.

Because CDK2-cyclin E plays a key role in the initiation of centrosome duplication, the proteins that control the CDK2/cyclin E activity are also expected to be critically involved in the regulation of centrosome duplication. The p53 tumor suppressor protein and its transactivation target p21$^{Waf1/Cip1}$ (p21) CDK inhibitor are the well-known regulatory proteins of the CDK2 activity (Sherr and Roberts, 1999). The involvement of p53 in the regulation of centrosome duplication was initially recognized by the observations that cells and tissues from p53-deficient mice show a high frequency of centrosome amplification resulting from overduplication of centrosomes (Fukasawa et al. 1996, 1997). The subsequent studies have revealed how p53 participates in the regulation of centrosome duplication. p53 and p21 are known to present at a basal level in cycling cells, monitoring untimely activation of CDK2-cyclin E in early to mid G1 phase (Minella et al. 2002; Nevis et al. 2009). When cyclin E expression is induced at late G1, the concentration of active CDK2-cyclin E complexes rapidly increases to the level beyond the capacity of the p53-p21 monitoring system, leading to initiation of centrosome duplication as well as DNA replication. Indeed, overexpression of exogenously introduced cyclin E in cells harboring wild-type p53 (and thus, continual activation of CDK2-cyclin E beyond the capacity of the p53-p21 monitoring system) results in initiation of centrosome duplication in early G1 phase (Mussman et al. 2000). In the absence of p53, p21 cannot be transactivated, hence allowing fortuitous activation of CDK2-cyclin E in early and mid-G1. Because Rho-bound ROCK II are already available in early G1, CDK2-cyclin E

prematurely triggers initiation of centrosome duplication through phosphorylation of NPM/B23 and consequential superactivation of ROCK II. However, because initiation of DNA replication requires many CDK2-cyclin E-independent events, CDK2-cyclin E can trigger DNA replication only after those events are completed, and thus the presence of active CDK2-cyclin E shortens the G1 duration for only few hours (Dulic et al. 1992; Koff et al. 1992). Thus, loss or mutational inactivation of p53 leads to uncoupling of initiation of centrosome duplication and DNA replication (Fig. 10.2). However, because uncoupling of initiation of centrosome duplication and DNA replication in cells lacking functional p53 depend on occurrence of "accidental" premature activation of CDK2-cyclin E, apparently the cells lacking functional p53 do not always experience the uncoupling of these two events, but in a long term, the majority of the p53-negative cells in a given population will experience uncoupling of centrosome duplication and DNA replication.

10.3 Loss of p53 and Centrosome Amplification

As described in other chapters, centrosome amplification leads to a high frequency of mitotic spindle defects and consequentially chromosome segregation errors. Centrosome amplification occurs frequently in various types of cancers, and is thought to be the major cause of chromosome instability in cancer cells (D'Assoro et al. 2002; Fukasawa 2005). Initially, induction of centrosome amplification by loss of p53 was identified in cells and tissues of p53-deficient mouse (Fukasawa et al. 1996, 1997). The mechanism of how loss of p53 leads to centrosome amplification was explored by the experimental system often referred to as "centrosome amplification (reduplication) assay", in which centrosomes undergo multiple rounds of duplication exposed to DNA synthesis inhibitors such as the DNA polymerase inhibitor (i.e., aphidicolin) and ribonucleotide reductase inhibitors (i.e., hydroxyurea (HU)), resulting in generation of amplified centrosomes. However, centrosome reduplication in the cell cycle-arrested cells occurs efficiently only when p53 is either inactivated or lost (Tarapore et al. 2001). In normal cells, p53 is upregulated in response to the physiological stress associated with the prolonged arrest by the ARF-mediated inhibition of Mdm2 (Sherr 2006) as well as DNA damages inflicted by the inhibitors by ATM/ATR- as well as Chk1/Chk2-mediated phosphorylation (Taylor and Stark 2001), leading to an increase in the intracellular level of p21, which in turn inhibits CDK2. Without the activity of CDK2, centrosome reduplication cannot be initiated. In contrast, in cells lacking functional p53, there will be no inhibitory mechanism for the CDK2 activity in response to the physiological and genotoxic stresses, and fortuitous activation of CDK2 leads to centrosome reduplication. This observation helped understanding the mechanism of how loss of p53 could lead to centrosome amplification (Fig. 10.3). Even under a normal growth condition/environment, cells are constantly subjected to internal as well as external stresses that temporarily halt cell
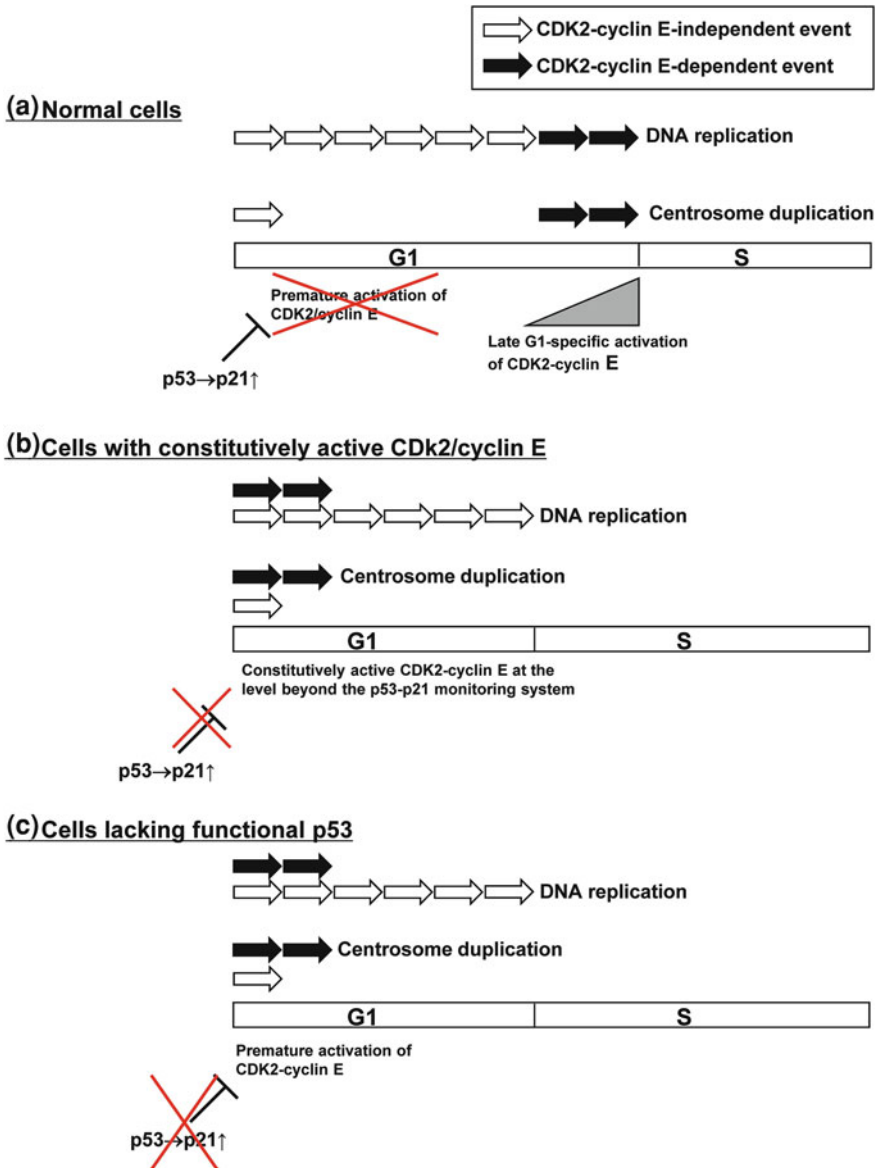
cycling irrespective of the p53 status (i.e., imbalance or deprivation of critical molecules such as dNTPs similar to the situation experimentally induced by HU treatment). In such cases, centrosomes reduplicate if cells lack functional p53, leading to centrosome amplification. Once the stress-causing problems are resolved, those cells resume cell cycling with amplified centrosomes.

◄**Fig. 10.2** The p53-p21 pathway monitors the premature activation of CDK2-cyclin E during G1 phase to ensure the coordinated initiation of centrosome duplication and DNA replication. CDK2-cyclin E is a triggering factor for both DNA replication and centrosome duplication. In normal cells, the basal levels of p53 and its transactivation target p21 monitor the premature activation of CDK2-cyclin E. In late G1, CDK2-cyclin E is activated by a temporal increase in cyclin E expression to the level beyond the p53-p21 monitoring capacity, and trigger initiation of both centrosome duplication and DNA replication (**a**). Initiation of DNA replication requires many CDK2-cyclin E-independent cellular events (*white arrows*) in addition to the CDK2-cyclin E-dependent events (*black arrows*). In contrast, initiation of centrosome duplication requires only few CDK2-cyclin E-independent cellular events (i.e., activation of ROCK II by Rho-binding) in addition to the CDK2-cyclin E-dependent events. Thus, constitutive activation of CDK2-cyclin E to the level beyond the p53-p21 monitoring capacity by cyclin E overexpression, centrosomes initiate duplication rapidly, while initiation of DNA replication occurs only after the completion of the CDK2-cyclin E-independent events. Thus, initiation of centrosome duplication and DNA replication is uncoupled (**b**). In the absence of p53, there would be no monitoring function to prevent premature activation of CDK2-cyclin E, and fortuitous activation of CDK2-cyclin E in early to mid G1 rapidly triggers centrosome duplication, but not DNA replication until the CDK2-cyclin E-independent events are completed. Thus, if p53 is lost or inactivated, cells experience uncoupling of initiation of centrosome duplication and DNA replication (**c**)

In mouse cells, loss or inactivation of p53 alone is sufficient to induce centrosome amplification at high frequencies (Fukasawa et al. 1996; Wang et al. 1998). However, it is not the case for human cells; inactivation/depletion of p53 in human primary fibroblasts by either expression of the dominant negative mutant p53 or small interfering RNA sequence targeting p53 does not efficiently induce centrosome amplification (Duensing et al. 2000; Bunz et al. 2002; Kawamura et al. 2004). Human cells are known to differ from mouse cells in the degrees of stringency in the regulation of cyclin E expression (Botz et al. 1996; Ekholm et al. 2001). In human cells, cyclin E expression is more strictly controlled than mouse cells, and occurs in a narrow window of late G1, and when cell cycle progression is halted, the activity of CDK2-cyclin E is tightly suppressed, and thus centrosome duplication remains blocked (Kawamura et al. 2004). In contrast, in mouse cells, cyclin E expression is relatively promiscuous, often showing the increased levels of cyclin E and active CDK2-cyclin E in the early-mid G1 (Mussman et al. 2000). In the absence of p53, the untimely activated CDK2-cyclin E is free from the p21-mediated inhibition, and triggers centrosome reduplication. In support of this scenario, loss of p53 together with cyclin E overexpression efficiently and syn-ergistically induces centrosome amplification in human cells (Kawamura et al. 2004). These observations explain why the studies examining human cancer tissues have repeatedly shown conflicting results for the association between p53 mutation and chromosome instability (or centrosome amplification); while many studies detected a positive association between p53 mutation and chromosome instability/centrosome amplification, many failed to do so. The finding of the synergistic actions of p53 mutation and cyclin E overexpression for induction of centrosome amplification in human cells suggests that centrosome amplification (and consequential chromosome instability) by p53 mutation in tumors can be profoundly affected by the status of cyclin E expression and the activity of CDK2-
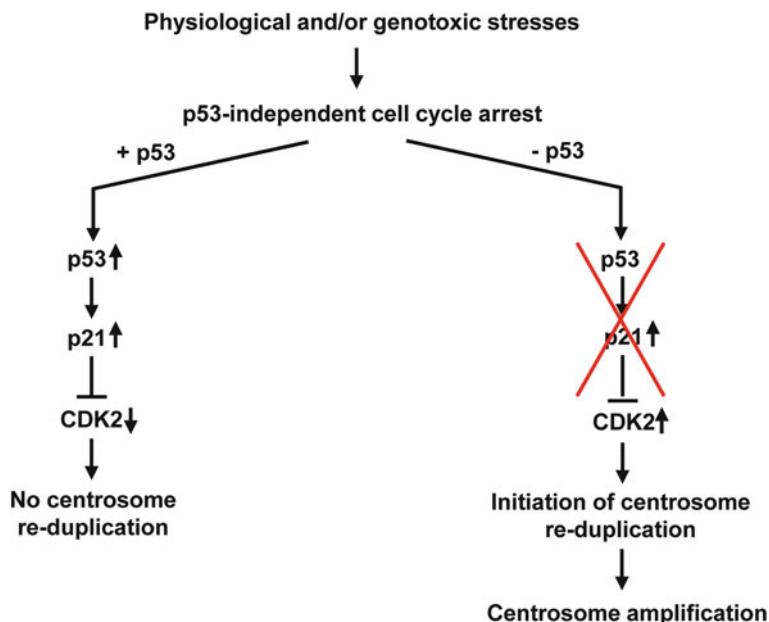
Fig. 10.3 Loss of p53 and centrosome amplification. Cells in any given populations and even under the optimal growth conditions are subjected to physiological and genotoxic stresses, resulting in the cell cycle arrest in a p53-independent manner. In cells with functional p53, p53 is upregulated by various mechanisms during the arrest. p53 then upregulates p21, which effectively inhibits CDK2. Without the activity of CDK2, newly duplicated centrosomes cannot undergo reduplication. In contrast, in cells lacking functional p53, p21 cannot be upregulated during the arrest, and if the active CDK2 is available, newly duplicated centrosomes undergo reduplication, resulting in centrosome amplification. Once the stress-causing problems are resolved, those cells will resume cell cycling with amplified centrosomes

cyclin E. The careful examination of bladder cancer specimens has revealed that this is indeed the case (Kawamura et al. 2004). This study shows that the occurrence of centrosome amplification parallels with increased frequencies of p53 mutation and cyclin E overexpression, and the multivariate analysis of the bladder cancer specimens in respect to status of p53, cyclin E expression and chromosome instability/centrosome amplification shows that there is a strong association between concomitant occurrence of p53 mutation and cyclin E overexpression and chromosome instability/centrosome amplification, but p53 mutation or cyclin E overexpression alone is not significantly associated with chromosome instability/centrosome amplification. Because cyclin E overexpression is frequent in many types of tumors (Keyomarsi and Herliczek 1997), the univariate analysis of p53 and chromosome instability/centrosome amplification tends to give a positive association. However, if cyclin E overexpression is a rare event in the tumor types under examination, the association between p53 mutation and chromosome instability/centrosome amplification will likely be weak. In support, it has been

shown that upregulation of E2F activity, which can be equated to the activation of CDK2-cyclin E, synergistically induces chromosome instability (aneuploidy) with p53 mutation in lung carcinomas (Karakaidos et al. 2004). Moreover, considering that chromosome instability is the driving force for acquisition of more malignant phenotypes, it is also consistent with the earlier studies showing that concomitant occurrence of cyclin E overexpression and p53 mutation strongly correlates with poor prognosis of renal pelvis, ureter, and gastric carcinomas (Furihata et al. 1998; Sakaguchi et al. 1998).

10.4 Other CDK2 and p53 Modulators and Centrosome Amplification

Because of the involvement of p53 in the regulation of centrosome duplication, the proteins that control the stability of p53 is expected to participate in the regulation of centrosome duplication, and aberrant expression/activity of such proteins leads to centrosome amplification. For instance, Mdm2, an E3 ubiquitin ligase that promotes degradation of p53 (Haupt et al. 1997; Kubbutat et al. 1997), is frequently overexpressed in various types of cancers, especially in those retaining wild-type p53 (Momand and Zambetti 1997). When MDM2 is overexpressed in mouse cells harboring wild-type p53, the level of p53 decreases, leading to efficient induction of centrosome amplification (Carroll et al. 1999).

Besides the p53-p21 pathway, the activity of CDK2 is also controlled by other CDK inhibitors, including p27^{Kip1} and p16(INK4a). Both p27^{Kip1} and p16(INK4a) have been implicated in the regulation of centrosome duplication. For example, centrosome amplification associated with DNA damage requires downregulation of p27^{Kip1} in certain cell types such as neuroblastoma cells (Sugihara et al. 2006). Loss of p16(INK4a) has also been shown to induce centrosome amplification (McDermott et al. 2006). In both cases, uncontrolled activation of CDK2-cyclin E was detected, which likely contributes to generation of amplified centrosomes.

10.5 Conclusion

It has been known that centrosome duplication occurs in coordination with other cell cycle-associated events. Thus, it is logical to predict that there are pathways that link centrosome duplication and the other cell cycle-associated events, including the RTK activation and DNA replication, and recent studies have started to identify those pathways. Regarding the molecular link between the RTK activation and centrosome duplication, the roles of the Rho-ROCK II pathway and STAT pathway were mainly discussed. However, the activated RTKs transmit the cell cycle signals to many downstream pathways, and other pathways may play equally important roles to link the growth stimulation and centrosome duplication, which remains to be determined

in the future studies. As described in this chapter, the key cell cycle-associated events, including centrosome duplication, are linked by the common regulatory proteins and pathways, and that many of those regulatory proteins are oncogenic and tumor suppressor proteins frequently mutated in cancers (Fukasawa 2007). Mutational activation or inactivation of those regulatory proteins can lead to uncoupling of centrosome duplication from other cell cycle-associated events, which lays a ground for occurrence of centrosome amplification, and consequentially destabilization of chromosomes, and thus profoundly influences the tumor development.

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