Review

Virologie

Virologie 2019, 23 (5) : E16-E31

Viral oncogenesis and genomic instability: the centr(osom)al connection

Oncogenèse virale et instabilité génomique : la connexion centr(osom)ale

Elodie Teruel[1,2]
Henri Gruffat[3]
Massimo Tommasino[4]
Chloé Journo[1,2]

[1] International Center for Research in Infectiology,
Retroviral Oncogenesis Laboratory,
INSERM U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308,
École Normale Supérieure de Lyon,
Université de Lyon, Lyon, France
[2] Equipe labellisée "Fondation pour la Recherche Médicale", France
[3] International Center for Research in Infectiology,
Oncogenic Herpesviruses Laboratory,
INSERM U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308,
École Normale Supérieure de Lyon,
Université de Lyon, 46 allée d'Italie,
69007 Lyon, France
[4] International Agency for Research on Cancer, Infections and Cancer Biology Group,
150 cours Albert-Thomas,
69008 Lyon, France

Abstract. Currently, more than 10% of human cancers are associated with viral infection. Studies on oncoviruses led to the development of clinical intervention strategies and elucidated fundamental cellular events altered upon cell transformation. Cancer cells exhibit several hallmarks including genomic instability, defined as a high frequency of mutations including gain or loss of chromosomes. The centrosome is an organelle that governs mitotic chromosome segregation and that functions as a signaling platform downstream of the DNA damage response. Here, we review the current literature to highlight how oncoviruses induce genomic instability *via* the deregulation of the centrosome. Viral interference with the centrosome duplication cycle, leading to centrosome amplification, is illustrated, with a special emphasis on mechanisms shared by several viral families. In addition, we discuss how oncoviruses could alter the signaling functions of the centrosome, and we comment on the bibliographic gaps that could be addressed by future research.

Key words : oncogenic viruses, centrosome amplification, cell cycle, DNA damage, aneuploidy

Résumé. A l'heure actuelle, plus de 10 % des cancers humains sont associés à une infection virale. L'étude des virus oncogènes a permis le développement de stratégies d'intervention clinique efficaces ainsi que l'élucidation fondamentale de processus altérés au cours de la transformation cellulaire. Cette dernière correspond à l'acquisition de caractéristiques cellulaires spécifiques incluant l'instabilité génomique, définie comme une fréquence élevée de mutations et de gains ou de pertes de chromosomes. Le centrosome est un organite qui gouverne la ségrégation des chromosomes au cours de la mitose et qui constitue une plateforme de signalisation en aval de la détection des dommages à l'ADN. Dans cette revue, nous proposons une synthèse de la littérature démontrant le rôle du centrosome dans la perte de l'intégrité génomique induite par les virus oncogènes. Nous illustrons les mécanismes par lesquels les virus oncogènes interfèrent avec le cycle de duplication du centrosome et induisent son amplification, en nous attachant à dégager les mécanismes communs à diverses familles virales. Nous discutons également de la façon dont une altération par les virus oncogènes des fonctions de signalisation du centrosome pourrait contribuer à l'instabilité génomique des cellules infectées, tout en soulignant les lacunes bibliographiques qui pourraient être comblées par les recherches à venir.

Mots clés : virus oncogène, amplification du centrosome, cycle cellulaire, dommages à l'ADN, aneuploïdie

Corresponding : C. Journo
<chloe.journo@ens-lyon.fr>

doi:10.1684/vir.2019.0793

To cite this article: Teruel E, Gruffat H, Tommasino M, Journo C. Viral oncogenesis and genomic instability: the centr(osom)al connection. *Virologie* 2019; 23(5):16-31 doi:10.1684/vir.2019.0793

Introduction

In 1911, the identification of Rous sarcoma virus as the first animal oncogenic virus (or oncovirus) provided the first evidence for the involvement of an infectious agent in cancer development. This discovery paved the way to the establishment of the link between several viral infections and human cancers. More than fifty years ago, Epstein-Barr virus (EBV) was identified as the first human oncovirus from a Burkitt lymphoma-derived cell line [1]. It is now well established that approximately 12% of human cancers are caused by oncoviruses [2, 3]. Seven human oncogenic viruses have been identified so far. Among DNA oncoviruses, EBV (now also known as human herpes virus type 4, HHV-4) is associated with B cell malignancies as well as epithelial malignancies. The Kaposi sarcoma-associated herpesvirus (KSHV, also known as human herpes virus type 8, HHV-8) also belongs to the *Herpesviridae* family and was first detected in Kaposi sarcoma associated to acquired immune deficiency syndrome (AIDS) [2]. Hepatitis B virus (HBV), one of the etiological agents of hepatocellular carcinoma, belongs to the *Hepadnaviridae* family. Approximately 12 human papillomaviruses (HPVs, *Papillomaviridae* family) that infect the mucosal epithelia of the genital and upper respiratory tracts, referred to as mucosal high-risk (HR) HPV types, have been clearly associated with anogenital cancers and with a subset of oropharyngeal cancers. In particular, HPV-16 and -18 are the most oncogenic HR types, being detected in approximately 50% and 20% of worldwide cervical cancers, respectively [2, 3]. Merkel cell polyomavirus (MCPyV) belongs to the *Polyomaviridae* family and is so far the only human polyomavirus known to be involved in tumorigenesis [4]. Some human oncoviruses also belong to RNA virus families. It is the case for hepatitis C virus (HCV), identified in the 1980's following sequencing analyses of transfusion-associated hepatitis cases that were not due to hepatitis A nor B viruses [5]. HCV is classified in the *Flaviviridae* family and is associated with hepatocellular carcinoma. Human T-cell Leukemia virus type 1 (HTLV-1) belongs to the *Retroviridae* family and is the etiological agent of adult T-cell leukemia/lymphoma (ATL) [6].

Since the mid-20th century, tumor virologists have been postulating that investigating the interactions between oncoviruses and infected cells would help to better understand the general process of cell transformation (reviewed in [7]). Cancer cells exhibit specific hallmarks such as aberrant cell cycle progression and cell proliferation, activated telomerase, inhibition of apoptosis, metabolic reprogramming, and genomic instability. The centrosome, a subcellular organelle that is commonly defined as the microtubule-organizing center (MTOC) in mammalian cells, allows the assembly of the mitotic spindle during cell division, and centrosomal defects are intimately linked to the promotion of genomic instability (for a detailed review on centrosome functions, see [8]). While the general process of genomic instability induction by oncoviruses has been reviewed elsewhere (see for instance [9]), here we will focus on the role of the centrosome in the loss of genomic integrity induced by oncoviruses. A particular focus will be given to the viral interference with the centrosome duplication cycle and, consequently, with chromosome segregation upon cell division. In the last decade, the centrosome has also emerged as a key integrator of signals downstream the DNA damage response (DDR). Thus, we will also discuss how oncoviruses could alter the centrosome functions in the context of the DDR.

A short introduction to centrosome structure and dynamics

The centrosome is composed of two orthogonal 500-nm long and 250-nm wide centrioles, referred to as the mother or daughter centriole, respectively, surrounded by a matrix of proteins known as the pericentriolar material (PCM, *figure 1*). At the proximal end, a centriole displays nine triplets of microtubules arranged to form a cylinder. The mother centriole is a mature structure with distal and sub-distal appendage involved in anchoring microtubules, whereas the daughter centriole is an immature structure with no appendage. The PCM initiates microtubule nucleation on γ-tubulin ring complexes, accounting for the MTOC function of the centrosome. Until recently, the PCM was viewed as an anamorphous protein matrix, but recent studies using super-resolution microscopy such as 3D-structured illumination microscopy have shown that the PCM is in fact a well-organized matrix of dynamically interacting proteins [10-12].

Together with chromosomes, the centrosome is the only cellular structure to be precisely duplicated and separated during each cell cycle. Similar to DNA replication, centrosome duplication is a semi-conservative process occurring once per cell cycle and is closely linked to the cell cycle progression. This allows cells entering mitosis to display two distinct centrosomes, which migrate at the opposite poles of the cell and induce the formation of a bipolar mitotic spindle. Upon mitosis, the two centrosomes are equally segregated and a daughter cell harbors only one centrosome (*figure 2*).

To ensure the duplication event, the mother and the daughter centrioles must dissociate and lose their orthogonal arrangement. This event is called centriole disengagement and is required for the nucleation of new procentrioles. The process of centriole disengagement is thus viewed as a

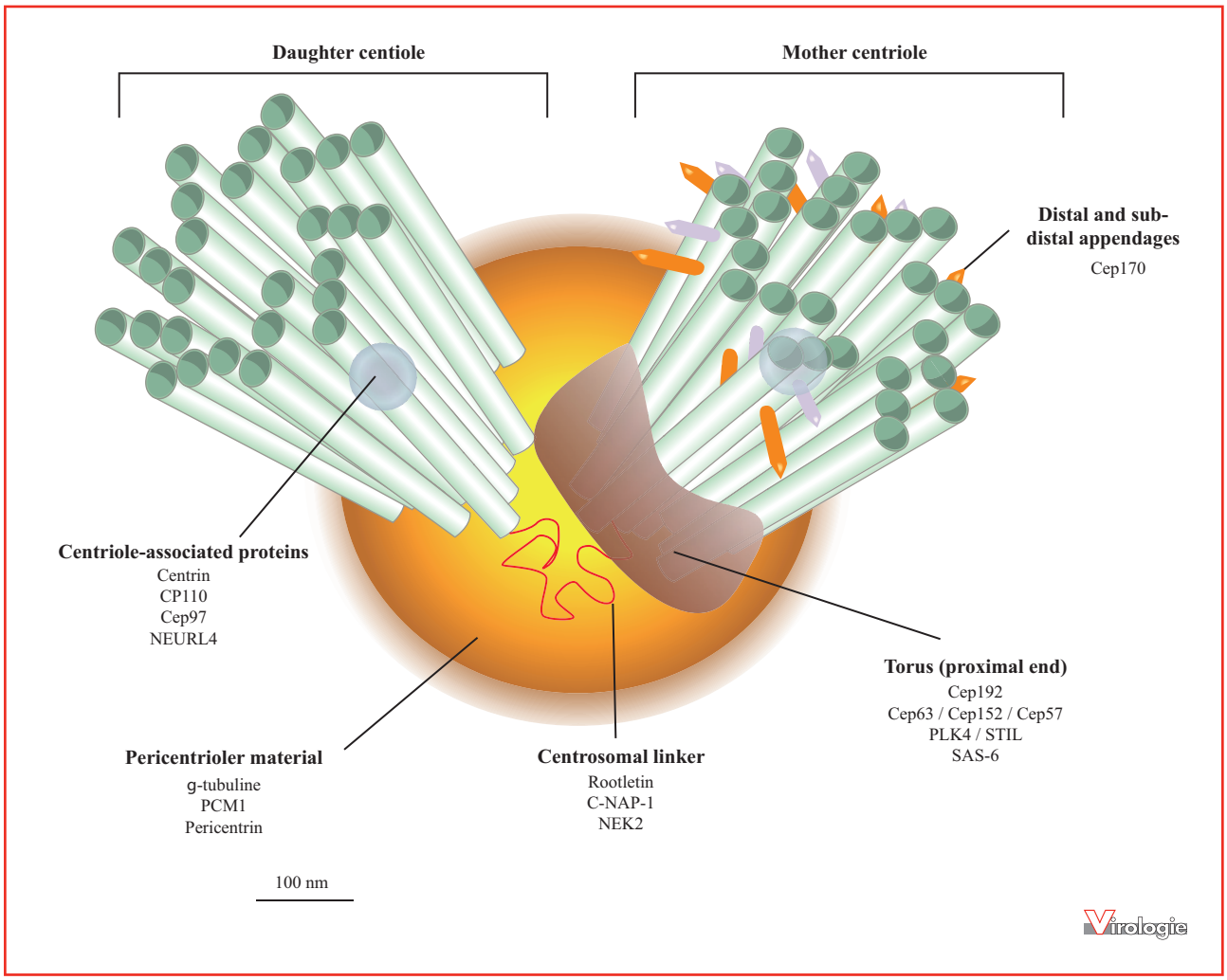

Figure 1. The centrosome structure. The centrosome is composed of two orthogonal centrioles (in green), each made of nine triplets of microtubules. In contrast to the daughter centriole (here on the left-hand side), the mother centriole (on the right-hand side) displays appendages at its distal end that are involved in anchoring microtubules (not represented). Both centrioles are linked by a centrosomal linker (in red) composed of rootletin and C-NAP-1 and regulated by NEK2, and are embedded in a well-organized protein matrix referred to as the pericentriolar material (yellow-to-brown gradient). Procentriole nucleation relies on the sequential assembly of Cep192, Cep63, Cep152, Cep57, PLK4, STIL and SAS-6 at the proximal end of the mother centriole (torus, in dark red). Centriole-associated proteins are represented as a blue sphere. For the sake of clarity, centriolar satellites have been omitted.

licensing step allowing subsequent duplication. Two key regulatory proteins, *i.e.* the mitotic kinase PLK1 and the separase protease, promote centriole disengagement in late M/early G1, together with the disassembly of mitotic PCM (*figure 2*, step 1). The separation of paired centriole has further been shown to be controlled by the G1/S phase cyclin A and cyclin E-activated CDK2 kinase. In this process, nucleophosmin (NPM1) has been identified as a substrate of CDK2 [13] that in turn activates ROCK II, an effector of Rho small GTPase, to promote centriole separation [14].

After centrioles are disengaged, a centrosomal linker composed of rootletin and C-NAP-1 connects their proximal ends throughout the interphase, as each centriole nucleates a new procentriole (*figure 2*, step 2). Procentrioles are oriented orthogonally to their mother centrioles and are elongated through the S and G2 phases until they reach the same length as the mother centrioles (*figure 2*, step 3). Ensuring that a single duplication event happens per cell cycle implies a precise regulation of the different factors that act sequentially at the proximal end of the mother centriole to promote procentriole nucleation, such as Cep192, Cep63, Cep152, Cep57, PLK4, STIL and SAS-6 (represented as the torus on *figure 1*, recently reviewed in [15]). Of note, the protein levels of these master

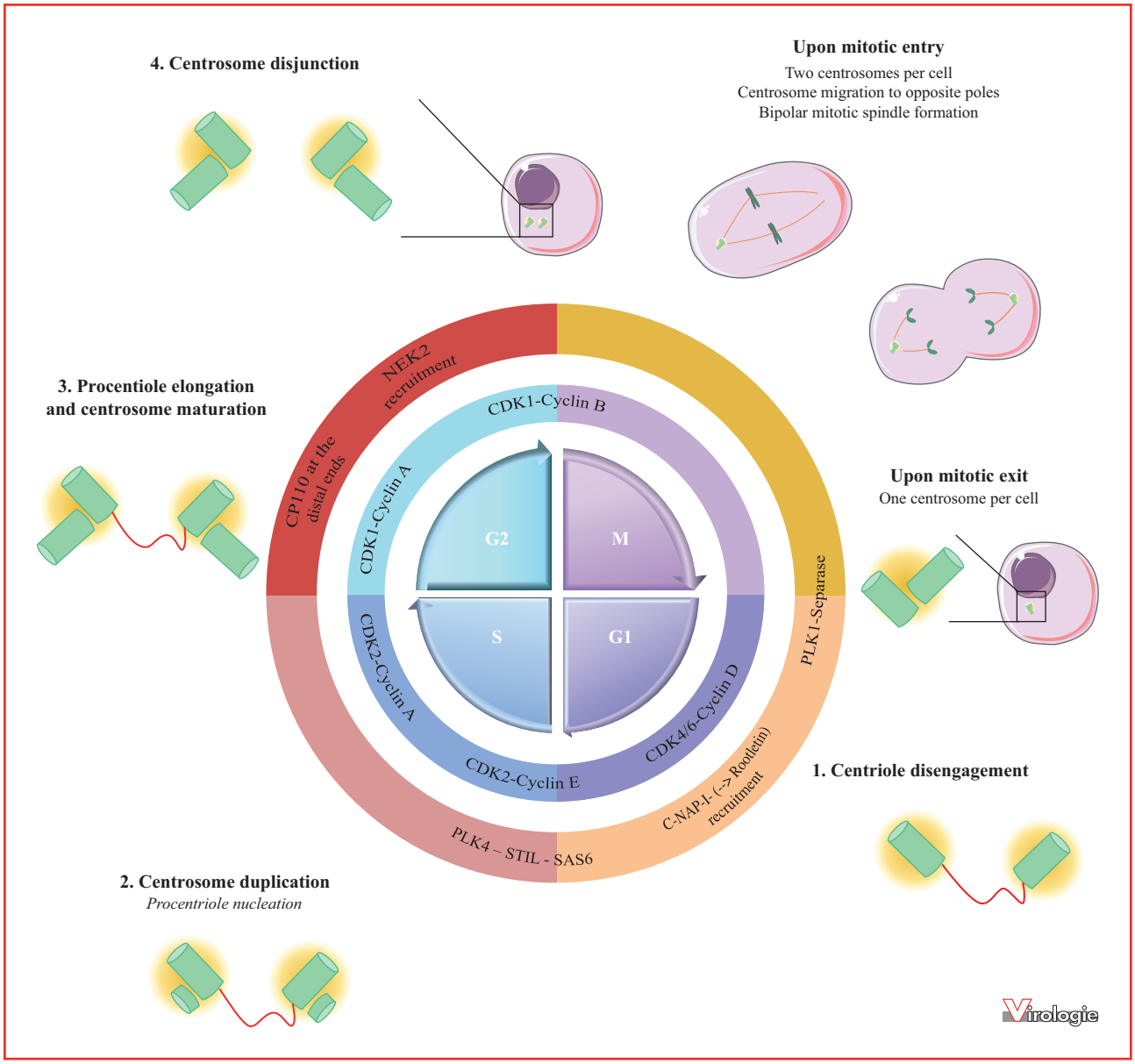

Figure 2. The centrosome duplication cycle. Upon mitotic exit, each cell harbors one centrosome. Centrosome duplication begins in late M/G1 phase with the physical disengagement of the two centrioles (step 1), which remain tethered by the linker (in red). As DNA replication occurs in S phase, the centrosome duplication *per se* starts with the nucleation of procentrioles at the proximal end of each parental centriole (step 2). Elongation of centrioles occurs in G2 phase, while centrosome maturation (shown as yellow to orange coloring) starts in G2 phase and continues into M phase (step 3). In late G2, the duplicated centrosomes undergo disjunction (step 4) and migrate to opposite poles to form the bipolar mitotic spindle in M phase (purple background). The main molecular regulators of the centrosome duplication cycle (outer circle) as well as the CDK/Cyclin complexes active during cell cycle progression (inner circles) are specified.

regulators, and in particular of PLK4, STIL and SAS-6, are tightly controlled by the ubiquitin-proteasome system. For instance, the downstream SAS-6 factor is a substrate of the SCF^{FBXW5} E3 ubiquitin ligase. SCF^{FBXW5} activity is inhibited by PLK4, stabilizing SAS-6 and allowing procentriole nucleation. After procentrioles are nucleated, PLK4 ubiquitinylation by the $SCF^{\beta\text{-}TrCP}$ E3 ubiquitin ligase, followed by its degradation, alleviates SCF^{FBXW5} inhibition and triggers SAS-6 degradation, thus limiting centriole overduplication. In addition to these master

regulators, a number of proteins cooperate to ensure the proper duplication cycle of the centrosome and the formation of functional MTOCs. For instance, CP110, a centrosomal protein localized at the distal ends of centrioles (*figure 1*), is required for centriole elongation (reviewed in [16]), and is able to promote the formation of ectopic MTOCs when over-expressed, leading to aberrant mitotic spindles. In normal cells, CP110 activity is counter-balanced by the E3 ubiquitin ligase NEURL4, which ubiquitinylates CP110 and promotes its proteasomal degradation [17].

As the cell progresses towards the M phase, duplicated centrosomes recruit expanded PCM in a process referred to as centrosome maturation (*figure 2*, step 3). This mitotic PCM will then allow the formation of the mitotic spindle in M phase. In late G2, the NIMA-related NEK2 kinase promotes centrosome disjunction by phosphorylating rootletin and C-NAP-1 to allow their removal from the centrosomes (*figure 2*, step 4). Consequently, the two centrosomes separate at the G2/M transition and induce the formation of the bipolar mitotic spindle.

A recent development in the understanding of centrosome structure and dynamics has come from the thorough description of centriolar satellites, defined as 70-100 nm electron-dense, non-membranous particles located in the vicinity of the centrosome (recently reviewed in [18]). The first and major identified component of centriolar satellites is PCM1 (pericentriolar material 1), which is thought to scaffold the whole structure, but over a hundred proteins have now been identified at centriolar satellites. Loss of centriolar satellite integrity, such as disappearance, dispersion, reduction or accumulation, can be achieved experimentally but has also been associated with several diseases. Interestingly, centriolar satellites are involved in the transient storage of centrosome constituents such as Cep63 and Cep152, and in their transport to the duplicating centrosome, supporting the notion that centriolar satellites are active regulators of the centrosome duplication cycle.

Centrosome amplification: a step towards genomic instability

Chromosomal instability, defined as the gain or loss of chromosomes or fragments of chromosomes, leads to aneuploidy. It is an important aspect of genomic instability: the majority of human solid tumors and more than 75% of hematopoietic cancers are aneuploid [19]. Over a century ago, Theodor Boveri suggested that aneuploidy may arise from centrosome amplification [20], a view that has been validated since then (for a review, see [21]). Importantly, a recent study by Levine *et al.* showed that centrosome amplification was sufficient to promote initiation of tumorigenesis in a transgenic mouse model, independently of additional genetic alterations [22]. This established that centrosome amplification can drive tumorigenesis in mice, at least in some models, by promoting chromosomal instability. A cell that possesses more than two centrosomes at the beginning of mitosis may form a multipolar mitotic spindle that results in chromosome missegregation and in the production of a highly aneuploid and inviable progeny, a process described as mitotic catastrophe (*figure 3*, left panel). However, centrosome clustering may lead to the formation of a so-called pseudobipolar spindle, which induces mild levels of aberrant mitotic features such as anaphase bridges and lagging chromosomes (*figure 3*, right panel), giving rise to micronuclei in the daughter cell. These mild levels of chromosomal instability are thought to be compatible with the emergence of transformed cell clones, indicating that centrosome clustering might represent an adaptive strategy for cells with supernumerary centrosomes to progress on the way to cell transformation (for reviews, see [23, 24]).

In cancer cells, centrosome amplification has mostly been linked to the dysregulation of the centrosome duplication cycle. PLK4 over-expression was shown to induce aberrant centriole overduplication in *Drosophila* embryos [25]. This was confirmed in *in vivo* mouse models (see for instance [22, 26]), and also in human cells, in which PLK4 cooperates with CDK2 and SAS-6 for centriole overduplication [27]. In addition, centrosome amplification can be a consequence of premature centriole disengagement. For instance, experiments of mitotic delay in G2/M-arrested cells showed an increased rate of centriole disengagement and centrosome fragmentation in an APC/C and separase-dependent manner [28]. Centrosome amplification may also be an indirect consequence of cell cycle defects such as failure in completion of cytokinesis [29]. This gives rise to polyploid cells harboring supernumerary centrosomes.

Interestingly, the p53 tumor suppressor has been shown to limit centrosome overduplication [30, 31], but also to block proliferation and/or induce apoptosis in polyploid cells as well as in cells with supernumerary centrosomes [22, 29, 32, 33], by a mechanism that involves the PIDDosome and caspase-2 [34]. Loss of p53 function, which is common in cancer cells, may thus favor the onset and maintenance of centrosome amplification, and may synergize with centrosome amplification (*e.g.* induced by PLK4 over-expression) to induce tumorigenesis [35, 36]. Of note, Levine *et al* also showed that although p53 was not genetically altered upon spontaneous centrosome amplification-dependent tumorigenesis in mice, the expression levels of p53 target genes was diminished, indicating that p53 pathway alteration may be required for centrosome amplification-triggered tumorigenesis [22].

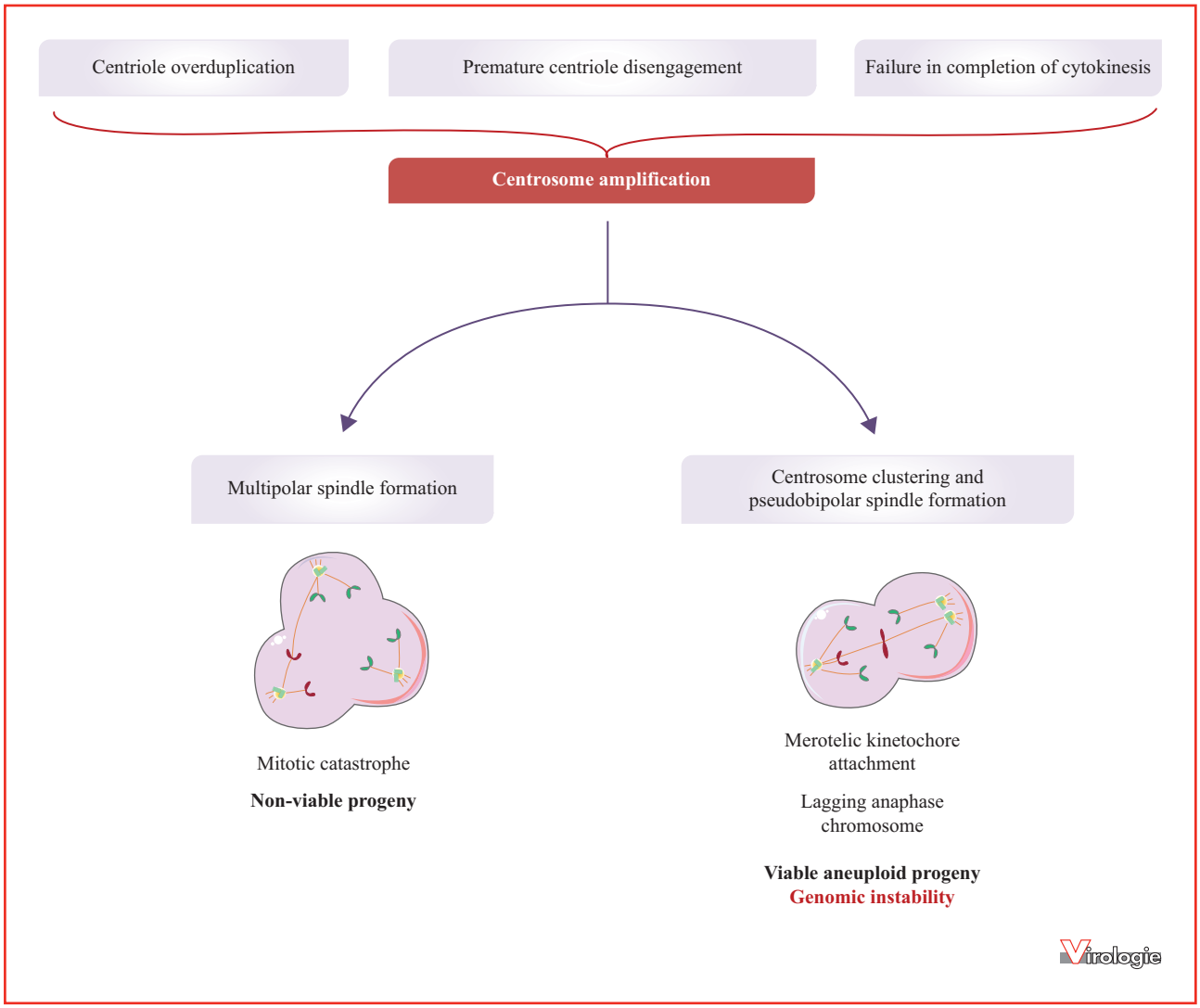

Figure 3. From centrosome amplification to genomic instability. Centrosome amplification can result from centriole overduplication, premature centriole disengagement, or failure in cytokinesis. Centrosome amplification may lead to multipolar divisions and mitotic catastrophe. Clustering of amplified centrosomes allows the formation of pseudobipolar divisions responsible for the induction of low-rate, viable aneuploidy.

Oncoviruses and centrosome amplification

Direct action of viral proteins on centrosome duplication

Centrosome amplification has been associated with infection by a number of oncoviruses. Some viruses have been shown to promote centrosome amplification by directly dysregulating proteins involved in the centrosome duplication cycle. For instance, expression of the HR HPV E7 oncoprotein induces centrosome amplification. A first study observed centrosome amplification in pre-invasive and invasive HPV-associated genital squamous lesions, and demonstrated that HPV-16 E7 stimulates centriole overduplication in normal human keratinocytes [37]. An independent analysis of cervical pre-invasive and invasive lesions corroborated these data and showed that the frequency of cells presenting centrosome amplification increases with the severity of HPV-associated lesions [38]. Mechanistic investigations demonstrated that E7 induces centriole overduplication through the transcriptional activation of Cyclin A and PLK4, as well as the aberrant activation of PLK4 by the CDK2/Cyclin E complex (*figure 4*)

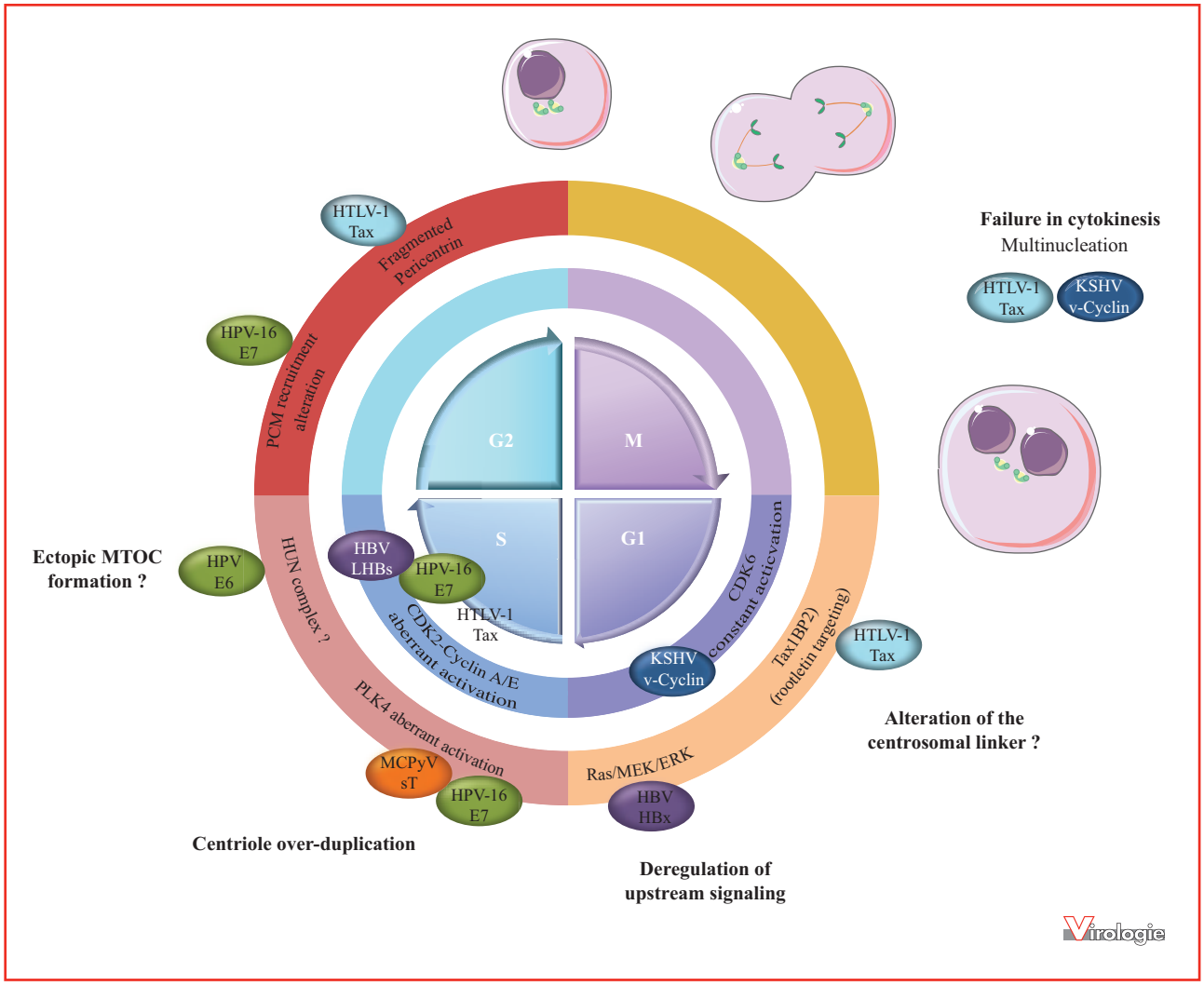

Figure 4. Centrosome amplification promoted by oncoviruses. Oncoviruses promote centrosome amplification through the direct or indirect dysregulation of cell cycle regulatory CDK/Cyclin complexes (inner circle) and alteration of the centrosome duplication cycle (outer circle), or by interfering with cytokinesis. Examples of viral oncoproteins promoting centrosome amplification are included to illustrate the common and specific targets of viral oncoproteins.

[39-43]. This results in the recruitment of an aberrant number of PLK4 dots at mother centrioles, allowing the nucleation of multiple procentrioles [43]. Interestingly, a similar phenotype of centriole overduplication is observed upon expression of MCPyV small T (sT) antigen (*figure 4*), which is sufficient to induce supernumerary centrosomes in NIH3T3 cells and in human fibroblasts, as well as to induce aneuploidy in transgenic mice [44]. This is dependent on the inhibition of cellular E3 ubiquitin ligase complexes such as SCF^{FBXW7} and $SCF^{\beta\text{-}TrCP}$ by sT [44]. These ligases target several key centrosomal regulators to proteasomal degradation, including Cyclin E and PLK4. Thus, HPV and MCPyV share the ability to induce centriole overduplication by aberrantly activating PLK4, although they use distinct mechanisms to promote PLK4 activation.

HR HPV types also appear to interfere with the centrosome duplication cycle *via* the HUN (HERC2, UBE3A, and NEURL4) complex [45]. Indeed, the UBE3A ubiquitin ligase, also known as E6AP (for E6-associated protein), was first identified as the target of HR HPV E6 proteins in the process of p53 ubiquitination and degradation. Interaction network analysis identified several centrosomal proteins as partners of UBE3A, such as CEP97, CEP170, and NEURL4 itself [45]. The fact that HPV may hijack the HUN complex for centrosome amplification was suggested, but it still remains to be clearly established. HR HPV E6 might indeed

induce UBE3A-dependent degradation of NEURL4, which would result in the stabilization of CP110 and the possible formation of ectopic MTOCs. Besides, HPV-16 E7 was shown to associate with centrosomes and to alter the recruitment of PCM to the centrosome [46], indicating that E7 could also directly alter centrosomal structure.

Centrosome amplification by direct interference with the centrosome duplication cycle is also a feature of HTLV-1 oncogenesis (for a review, see [47]). Indeed, the expression of HTLV-1 Tax protein is associated with centrosome amplification [48]. Tax-associated changes in centrosome morphology, such as a fragmented aspect for the PCM protein pericentrin, are also frequently observed in Tax-expressing cells [49]. Tax constitutively activates CDK2 (*figure 4*), suggesting that similar to HPV E7, a dysregulated CDK2 activity contributes to centrosome overduplication in HTLV-1 infected cells. In addition, Tax was also shown to target the centrosomal protein TAX1BP2, a splicing isoform of rootletin [50]. This suggests that Tax may abrogate TAX1BP2/rootletin functions to subvert the cellular control of centrosome duplication (*figure 4*).

Indirect action of viral proteins on upstream signaling

HBV encodes two viral oncoprotein, HBx and the large surface antigen LHBs, the latter often being mutated in the preS2 region in hepatocellular carcinoma patients. Two independent studies showed that the *in vitro* expression of HBx results in an increased frequency of cells with an abnormal number of centrosomes, correlated with the formation of aberrant mitotic spindles and chromosomal missegregation that subsequently increase the number of aneuploid cells [51, 52]. HBx-expressing cells fail to regulate centrosome duplication due to the activation of the upstream Ras-MEK-ERK pathway by HBx [52]. Expression of preS2-mutant LHBs was reported to induce centrosome overduplication by a mechanism that depends on the endoplasmic reticulum (ER) stress response and on calcium release [53]. ER stress was indeed shown to lead to the calpain-dependent cleavage of cyclin A. The truncated form of cyclin A still interacted with CDK2 and was suggested to be responsible for centrosome overduplication (*figure 4*) [53]. Whether ER stress also participates in centrosome amplification under other oncogenic stimulations (either associated to oncoviral infections or not) represents an intriguing hypothesis that warrants further investigation.

Indirect action of viral proteins on cytokinesis

Indirect centrosome amplification due to failure in cytokinesis has also been described for oncoviruses. HTLV-1 Tax interacts with the mitotic spindle assembly checkpoint protein MAD1, leading to the loss of MAD1 functions and to the emergence of multinucleated cells [54]. Because MAD1 was suggested to link mitosis with cytokinesis, abrogation of MAD1 functions by Tax might both inactivate the spindle assembly checkpoint and lead to failure in cytokinesis, which could contribute to the centrosome amplification observed in Tax-expressing cells. KSHV induces chromosomal instability by the expression of v-Cyclin, a viral cyclin D2 analog interacting with cellular CDK6 [55]. v-Cyclin promotes G1/S transition and allows DNA replication, while blocking cytokinesis (*figure 4*). Expression of v-Cyclin thus leads to polyploidization and centrosome amplification associated with multinucleation. This process requires CDK6 and its substrate NPM1 [32, 56-58]. Consistent with these observations obtained in v-Cyclin-expressing cells, centrosome amplification associated with multinucleation has been confirmed in KSHV-infected primary endothelial cells [58, 59].

These examples illustrate a long-standing question in the field of centrosome biology and cancer: are centrosomal defects a cause or a consequence of mitotic aberrations contributing to genomic instability? As illustrated here, primarily-induced mitotic defects drive secondary centrosome amplification, which can then amplify mitotic defects by generating multipolar or pseudobipolar mitotic spindles. Because oncoviruses often disrupt multiple cellular processes that cooperate in perturbing the cell cycle, whether centrosomal defects are a driving force in viral oncogenesis or rather appear as a secondary event is still a matter of debate.

Cooperation between centrosome amplification and p53 loss-of-function upon oncovirus infection

As stated above, loss of p53 function has been shown to synergize with centrosome amplification for tumorigenesis. Interestingly, it was through its interaction with the SV40 LT viral oncoprotein that p53 was first discovered in 1979 [60, 61], and the interplay between oncoviruses and p53 functions has now been extensively documented (for a recent review on human oncoviruses and p53, see [62]). For instance, several viral oncoproteins share the ability to induce p53 degradation, including KSHV LANA, HR HPV E6 and EBV BZLF1, while others such as HR HPV E6 and E7, HTLV-1 Tax and HBV HBx inhibit its transcriptional activity. However, the extent to which oncovirus-induced loss of p53 function is involved in the generation and maintenance of cells that harbor supernumerary centrosomes has not been systematically analyzed. One of the most convincing example of a cooperation between centrosome amplification and p53 loss-of-function upon oncovirus infection is the case of KSHV, for which centrosome amplification following multinucleation was shown to be

potentiated by the loss of p53 function [32, 56-58]. Centrosome amplification in HR HPV-associated lesions was also suggested to follow p53 functional inactivation [38], but the formal demonstration has been complicated by the fact that p53 inactivation and centrosome amplification are overlapping properties of HPV oncoproteins [63]. While the ability of HBV HBx protein to induce supernumerary centrosomes has been compared in WT and p53-deficient cells, the reported results are unclear and the experiments should be repeated [51]. Thus, delineating the requirement of p53 loss-of-function for oncovirus-induced centrosome amplification and subsequent induction or maintenance of aneuploidy remains a challenge for future work.

New regulators of centrosome organization and dynamics identified through the analysis of oncoviruses

Studying the impact of oncoviruses on centrosome amplification has also contributed to our understanding of regulatory mechanisms occurring in normal, uninfected cells. An example of such a contribution lies in the identification of the involvement of the Ran-GTPase network in centrosome organization and dynamics. The HBx oncoprotein from HBV was indeed shown to interfere with the Crm1/Ran GTPase-dependent nuclear export pathway, by sequestering Crm1 in the cytoplasm. This observation was then suggested to be causally linked to HBx-induced centrosome amplification, since an HBx mutant unable to sequester Crm1 did not amplify centrosomes [51]. Interestingly, a fraction of Crm1 as well as of Ran localizes at the centrosome in uninfected cells, and inhibition of Crm1/Ran function in uninfected cells leads to centrosomal aberrations, in the form of extra minicentrioles that lack PCM, or abnormally elongated centrioles [51]. The notion thus emerged that Crm1/Ran may prevent centrosome amplification. Because the Crm1/Ran pathway was known to shuttle cellular factors between the nucleus and the cytoplasm, it was hypothesized that the timely nucleocytoplasmic transport of regulators of centrosome duplication by Crm1/Ran, such as NPM1 or Cyclin B, might be critical to ensure a proper centrosome duplication cycle (for a review, see [64]). Centrin and pericentrin are also targets of Crm1/Ran [65], indicating that Crm1/Ran might regulate the nucleocytoplasmic transport of key centrosome components.

Of note, Ran has also been involved in the regulation of mitosis, independently of its function in nucleocytoplasmic shuttling. Ran was indeed shown to regulate the mitotic aster and spindle formation by modulating the functions of spindle assembly factors such as TPX2 and NuMA, in a so-called “gradient” model. In this model, active GTP-bound Ran is generated at chromatin and is converted to inactive GDP-bound Ran while diffusing away from the chromatin, thus building a Ran-GTP gradient that can provide a positional information for the assembly of the spindle around chromosomes (for a review, see [66]). In line with these Ran functions, RanBP1, a major Ran regulator, was also shown to generate multipolar spindles with monocentriolar poles when over-expressed, resulting from aberrant centriole splitting in mitosis [67].

An interaction between Ran and viral oncoproteins, *i.e.* HR HPV E7, adenovirus E1A and SV40 LT, has also been reported and associated with centrosome amplification [68]. HTLV-1 Tax was also shown to interact with RanBP1 at mitotic centrosomes [49]. Taken together, these observations driven by study of oncoviruses strengthen the idea that the Ran-GTPase network participates in the control of the centrosome dynamics and of the mitotic centrosome organization, and is a common target of viral oncoproteins (for a review, see [69]).

Oncoviruses with suspected actions on centrosome amplification

Induction of genetic instability associated with centrosomal alterations has not been extensively analyzed for the EBV and HCV oncoviruses. In an early study, the EBV enzyme thymidine kinase (TK) was shown to localize at the centrosome, but the significance of this observation relative to centrosome amplification, although discussed by the authors, remains elusive [70]. Centrosome amplification was nonetheless recently described in *in vitro* EBV-infected B cells and in an *in vivo* mouse model of EBV infection [71]. In this study, the EBV structural protein BNRF1, which is known for its role in the inhibition of cell host intrinsic defenses through its interaction with the host nuclear protein DAXX [72], was shown to localize at the centrosome and to be sufficient to promote centrosome amplification. However, its mechanism of action remains poorly understood. Supernumerary centrosomes have been described in cells expressing the HCV NS5A protein, which also delays mitotic exit, indicating that centrosome amplification might be a consequence of NS5A interference with mitotic regulation [73], as is the case for KHSV v-Cyclin. However, as for EBV BNRF1, HCV NS5A mechanism of action deserves further investigation.

An impact of oncoviruses on centrosome clustering?

As mentioned above, centrosome clustering is a prerequisite for cells with amplified centrosomes to give rise to a viable progeny. Centrosome clustering may be controlled by physical parameters. Indeed, spindle tension due to the incorrect attachment of kinetochores to microtubules arising from a multipolar spindle has been shown to induce the

repositioning of amplified centrosomes and their clustering [74]. In line with these observations, some cellular proteins involved in the positioning of the mitotic spindle and in the interaction between kinetochores and microtubules (including regulators of the spindle assembly checkpoint) have been shown to be required for centrosome clustering. These include components of the chromosomal passenger complex, of the Ndc80 microtubule-kinetochore attachment complex, of the sister chromatid cohesion complex and of the augmin complex [75]. Microtubule-associated proteins such as dynein and kinesin, as well as components of the acto-myosin cytoskeleton are also required for centrosome clustering [76, 77]. Interestingly, whether oncoviruses affect centrosome clustering has not been investigated in depth so far. Centrosome clustering has been observed in primary endothelial cells expressing the KSHV v-Cyclin protein [58], indicating that KSHV might not hinder centrosome clustering. However, whether the virus enhances centrosome clustering has not been determined. Because interaction of oncoviruses with components of the spindle assembly checkpoint has been well documented [54, 78-83], viral interference (either positive or negative) with centrosome clustering remains an open question that should be carefully analyzed in future experiments.

The centrosome as a signaling organelle for cell cycle checkpoints

The centrosomal initiation of mitosis

In addition to its role in chromosome segregation during mitosis, some studies argue that the centrosome plays an important checkpoint role in the initiation of M phase (for a review, see [84]). Indeed, signaling proteins implicated in the initiation of mitosis, including the master regulator complex CDK1/Cyclin B, the activating phosphatases Cdc25B/C, the mitotic PLK1 kinase and Aurora A, have been localized at the centrosome during the G2/M transition. CDK1/Cyclin B recruitment requires the centrosomal Cep63 protein [85]. Interestingly, Cdc25B was shown to activate CDK1/Cyclin B specifically at the centrosome [86]. The centrosome might thus be considered as a signaling platform that brings regulatory components into close proximity to ensure a correct timing of mitosis onset. Of note, HTLV-1 Tax was found to interact with Cep63 (our unpublished results) and might thus also interfere with the centrosomal control of the G2/M transition.

The centrosomal activation of cell cycle checkpoints in the DNA damage response

Furthermore, the centrosome has recently emerged as a signaling platform during the DNA damage response, which also plays a significant role in genomic instability prevention. DNA damage refers to changes in the basic structure of DNA that can occur naturally, *e.g.* during DNA replication, or that can be induced by exogenous agents. Depending on the insult, these changes can consist in mismatches, single strand breaks (SSBs), double strand breaks (DSBs) or stalled replication forks (resulting in junctions between single-stranded DNA and double-stranded DNA sensed as a replicative stress). If DNA damage is not properly repaired, it may contribute to the loss of genomic integrity. The DNA damage response (DDR) thus refers to an arsenal of strategies that prevent the accumulation of DNA damage, and includes damage sensing as well as the orchestration of the repair process together with the regulation of the cell cycle or the induction of apoptosis (reviewed in [87]). The DDR might activate one of three checkpoints: the G1/S checkpoint that prevents DNA replication in case of DNA damage, the S-phase checkpoint that stops DNA synthesis when damage occurs during replication, and the G2/M checkpoint that ensures that cells do not enter mitosis unless damage has been properly repaired.

ATM, ATR and DNA-PK sensor kinases are considered as the core of the DDR and respond primarily to DSBs for ATM and DNA-PK, and to replicative stress for ATR. Activation of these core proteins will be illustrated here with ATM activation (*figure 5*). Following DSBs, the MRN complex (Mre11, Rad50 and Nsb1) is directly recruited on the site of breakage and allows the autophosphorylation of inactive dimers of ATM at Ser1981, leading to their dissociation into active monomers [88]. Activated ATM phosphorylates the histone variant H2AX, then called γH2AX. γH2AX interacts with the MDC1 scaffold protein and recruits additional MRN complexes for a positive feedback loop on ATM activation. In addition to activating the repair machinery, ATM activates the G2/M checkpoint *via* the nuclear CHK2 transducer kinase (*figure 5*). By inhibiting Cdc25C phosphatase activity, activation of CHK2 ultimately leads to inhibition of the mitotic CDK1 kinase and activation of the cell cycle checkpoint. The related CHK1 kinase fulfills similar actions but is activated downstream of ATR.

Interestingly, ATM/ATR as well as CHK1 and CHK2 have been observed at the centrosome, indicating that the centrosome might function as a signaling platform in the DDR [89-91] (*figure 5*). Accumulation of CHK1 at the centrosome upon DNA damage leads to the inactivation of the CDK1/Cyclin B complex that is normally required for mitotic entry [92]. Activated CHK2 has also been reported to localize at the centrosome upon DNA damage [91]. Intriguingly, in unstressed cells, activated CHK2 is also associated to the centrosome, and more precisely to the mother centriole [91], raising the possibility that it could participate in regulating the centrosome duplication cycle in the absence of DNA damage.

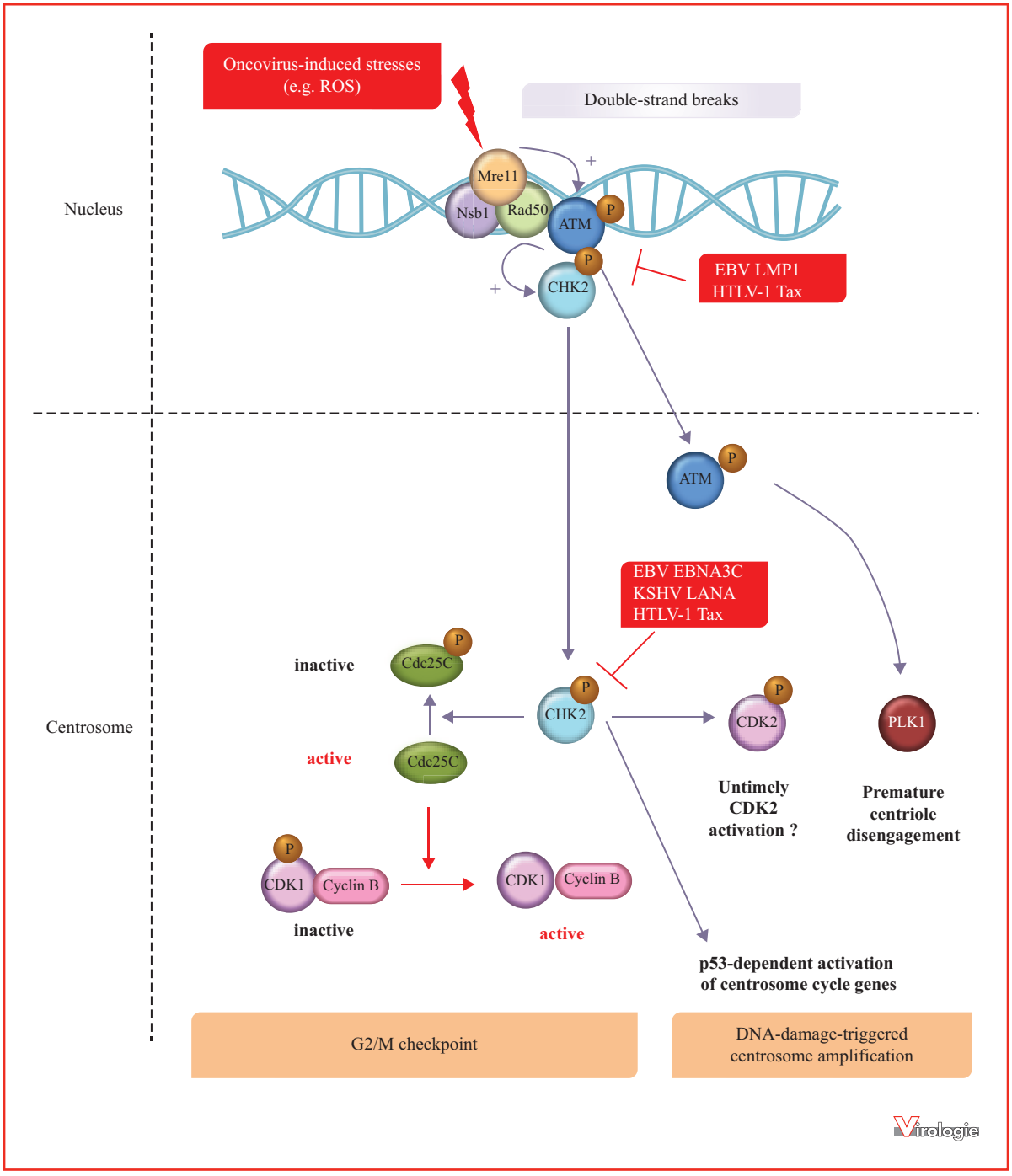

Figure 5. The centrosome at the interface between the cell cycle regulation and the DNA damage response. The activation of the G2/M checkpoint by double-strand breaks (DSBs) is illustrated. Upon DSBs, which can result from oncovirus-induced stresses, ATM activation occurs in the nucleus after the MRN complex (Mre11, Rad50 and Nsb1) is recruited at the site of the break. For the sake of clarity, the amplification loop involving γH2AX and MDC1 has been omitted. ATM activation leads to the phosphorylation of CHK2, which in turn inhibits Cdc25C phosphatase. This limits CDK1/Cyclin B activation for G2/M transition and results in cell cycle delay. ATM, CHK2 and downstream factors have been observed at the centrosome upon DNA damage. Oncoviruses disrupt these mechanisms mainly at the level of ATM and of CHK2, allowing CDK1/Cyclin B activation and checkpoint bypass (red boxes, arrows and text). Mechanisms of DNA damage-induced centrosome amplification are also illustrated.

The centrosome: a target of oncoviruses in the DNA damage response?

Most oncoviruses are known to favor genetic instability directly through DNA damage induction (mostly through the production of ROS), and indirectly through DDR impairment. Oncoviruses impair the DDR through the inactivation of sensor kinases and in particular of ATM (*figure 5*). As an example, EBV was shown to dysregulate ATM in Hodgkin's lymphoma. More precisely, the viral latent membrane protein LMP1 down-regulates the expression of ATM [93]. HTLV-1 Tax interacts with the Wip1 phosphatase and thereby mediates the dephosphorylation and inactivation of ATM, γ-H2AX and CHK1/2 [94]. Tax also forms a complex with MDC1 and DNA-PK in nuclear foci, thus impairing their involvement in the DDR.

Oncoviruses also interfere with downstream signaling pathways, leading to cell cycle dysregulation (*figure 5*). For instance, the G2/M checkpoint is disrupted in EBV-infected cells [95]. The EBNA3C viral protein interacts with CHK2, leading to a decreased CHK2 activity and to progression through the G2/M checkpoint. Similarly, the G2/M checkpoint is dysregulated through the action of the KSHV LANA protein. The HTLV-1 Tax oncoprotein induces CHK2 hyperphosphorylation, which results in CHK2 nuclear sequestration and to inhibition of its signaling activities [96].

Together with the observation that the centrosome might function as a signaling platform in the DDR, the fact that some viral oncoproteins known to interfere with the DDR also physically interact with the centrosome, such as HTLV-1 Tax, raised the possibility that these viral proteins could interfere with the DDR by specifically targeting the signaling activity of the centrosome. This hypothesis should be tested in future work.

Towards an integrated understanding of the centrosome dynamics: cross-talks between the DDR and the control of centrosome number

Several studies indicate functional cross-talks between the DDR and the centrosome amplification process that both have been linked to genetic instability. These cross-talks were first considered following the observation that drugs that induce DNA damage also induce centrosome amplification. For instance, hydroxyurea (HU) induces a replicative stress leading to a prolonged S phase through CHK1 and CHK2 activation [91], but also to supernumerary centrosomes. Inhibition of either CHK1 or CHK2 activity reduced centrosome amplification upon DNA damage-inducing treatments [91, 97], indicating that these kinases are required for DNA damage-triggered centrosome amplification. Because CDK2 is a substrate of CHK1/2, it might be a downstream effector of CHK1/2 activity involved in centrosome amplification (*figure 5*). DNA damage also induces an increase in the amount of PCM in a CHK1-dependent mechanism [98]. This PCM expansion requires the PCM protein pericentrin, and is exacerbated in the absence of microcephalin (MCPH1), a CHK1 interactor and regulator localized at the centrosome. Interestingly, inhibition of the ATM/CHK2 pathway leads to a reduced ability of KSHV to induce supernumerary centrosomes [58], indicating that centrosome amplification by KSHV might at least in part be a consequence of the DDR. Whether this holds true for other oncoviruses should be examined.

Premature centriole disengagement in G2 rather than in late M / early G1 phase, leading to centrosome re-duplication before mitosis, has also been observed as a consequence of DNA damage. The mitotic PLK1 kinase, which controls centriole disengagement, and which is a downstream target of ATM, has been involved in this premature DNA-damage-induced centriole disengagement (*figure 5*) [99]. Silencing C-NAP-1 and rootletin, which build up the centrosome tether, further increases centriole disengagement in G2 upon DNA damage [100], confirming the link between DNA damage and centriole disengagement.

Another observation that supports the notion of centrosome amplification being a consequence of the DDR is the fact that a number of genes involved in centrosome duplication, maturation or separation, such as Cep152 or NEK2, are transcriptionally activated following DNA damage (*figure 5*) [101]. Deficiency in p53 leads to an aberrant transcriptional regulation of genes involved in centrosome duplication [31, 102]. In addition, a fraction of p53 localizes at the centrosome, indicating potential functions in centrosome regulation [103].

DNA damage-induced centrosome amplification has recently been shown to arise from accumulation of centriolar satellites [104]. In this study, an elegant strategy using photoconvertible centrin-2 was used to distinguish between centrosome fragmentation and centrosome overduplication as a mode of centrosome amplification upon DNA damage. In these settings, no centrosome fragmentation could be observed, but centrosome amplification rather resulted from *de novo* centrin-2 assembly. These supernumerary centrin-2 dots were in fact identified as centriolar satellites, based on the observation that they were mobile and had a changeable morphology, and more importantly that they stained only weakly positive for *bona fide* centrosomal markers such as γ-tubulin, C-NAP-1, rootletin or SAS-6, but brightly positive for the centriolar satellite marker PCM1. Appearance of excessive satellites was further shown to allow *bona fide* centrosome amplification by promoting the transport of centrosome constituents.

Taken together, these observations indicate that centrosome amplification might arise as a consequence of DNA damage-activated checkpoints. Given that supernumerary centrosomes may lead to multipolar mitoses and induce mitotic catastrophe, DNA damage-induced centrosome amplification has been suggested to participate in the passive elimination of cells exhibiting prolonged and unrepaired DNA damage. However, cell processes have been described that counteract the above-mentioned mechanisms of DNA damage-induced centrosome amplification. Cep63 has for instance been shown to be a target of ATM and to impose a delay in mitotic progression upon DNA damage [105]. Recently, the centrosomal regulator TAX1BP2 was also described as a direct substrate of ATM [106]. Phosphorylation by ATM protects TAX1BP2 from ubiquitin-dependent proteasomal degradation and thus possibly enhances its ability to limit centrosome amplification upon DNA damage.

Since most oncoviruses induce DNA damage as discussed above, it is most probable that their association with centrosome amplification is at least partly dependent on these DNA damage-induced mechanisms. Of note, HTLV-1Tax interacts with both Cep63 and TAX1BP2 (see above), suggesting that oncoviruses may interfere with these molecular cross-talks between DNA damage and centrosome amplification. Delineating the direct impact of oncoviruses on the centrosome duplication cycle *versus* the indirect impact following DNA damage thus represents a challenging perspective for future work.

Conclusion and perspectives

Taken together, the data discussed in this review highlight the central role of the centrosome in the maintenance of genome integrity. While centrosome amplification as well as interference with the DDR are common features of infections by oncogenic viruses, how these two aspects of the loss of genome integrity interact at the centrosome to lead to cellular transformation remains to be investigated.

In the light of recent advances in the field of centrosome biology, centrosomal alterations induced by oncoviruses deserve to be revisited. In particular, structural aberrations in infected cells or in cells expressing selected viral proteins should be more carefully described, using state-of-the-art microscopy techniques. Many of the studies discussed above have indeed only used markers such as centrin-1 (a marker of centrioles) or γ-tubulin (a marker of the PCM) to track and count centrosomes by microscopy. However, such a use of individual markers might not be sufficient to distinguish between several types of structural aberrations that might affect the centrosome (e.g. overduplication, fragmentation, or accumulation of centriolar satellites). Combinatorial use of several markers of centrioles (e.g. mother *vs.* daughter centrioles, or proximal *vs.* distal end of centrioles) and of the PCM, together with markers of satellites, would allow a much more accurate description of structural aberrations, which in turn could be indicative of the underlying process targeted by oncoviruses. Such an in-depth description would also benefit from the recently developed semi-automated microscopy approaches that allow screening of multiple samples for centrosome aberrations [107].

In line with this need for revisiting oncovirus-induced centrosomal aberrations, recent methodological advances for the analysis of the centrosome structure and composition have proven profoundly informative and should be applied to infected cells or cells expressing viral oncoproteins. These advances include super-resolution microscopy [12] as well as innovative proteomic approaches such as proximity-dependent biotinylation (BioID) [108]. Adapting these approaches to systematically compare centrosome aberrations induced by distinct oncoviruses should allow identifying common targets of oncoviruses, and thus unravel common themes in viral-induced centrosomal dysfunctions. This could be informative as to which centrosomal factors or mechanisms are vulnerable to viral interference and hence, contribute to further characterize key regulatory networks involved in the loss of centrosomal integrity upon cell transformation.

Acknowledgments. We thank Veronique Marthiens and Karine Monier for insightful discussions on the biology of centrosomes. We also thank Renaud Mahieux and Hélène Dutartre for their critical proof-reading, as well as all colleagues for helpful discussions.

Conflicts of interest : This work was supported by an INSERM U1111 intramural grant to C.J. and H.G., and by the "Ligue Nationale contre le Cancer, Comité du Rhône, Projet Pluri-Equipes 2018" program to C.J., H.G. and M.T. C.J. is funded by the "ENS de Lyon". E.T. is supported by the "Ministère de la Recherche". Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer/World Health Organization.

References

1. Epstein MA, Achong BG, Barr YM. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. *Lancet* 1964 ; 28 : 702-3.

2. International Agency for Research on Cancer, Weltgesundheitsorganisation, eds. *IARC monographs on the evaluation of carcinogenic risks to humans, volume 100 B, biological agents*. IARC, 2012 [this publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 24 February-03 March 2009].

3. De Martel C, Ferlay J, Franceschi S, *et al*. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. *Lancet Oncol* 2012 ; 13 : 607-15.

4. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* 2008 ; 319 : 1096-100.

5. Choo Q-L, Kuo G, Weiner AJ, *et al*. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989 ; 244 : 359-62.

6. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci U S A* 1980 ; 77 : 7415-9.

7. Pipas JM. DNA tumor viruses and their contributions to molecular biology. *J Virol* 2019 ; 93 : e01524-18. doi: 10.1128/JVI.01524-18.

8. Conduit PT, Wainman A, Raff JW. Centrosome function and assembly in animal cells. *Nat Rev Mol Cell Biol* 2015 ; 16 : 611-24.

9. Yasunaga J, Jeang K-T. Viral transformation and aneuploidy. *Environ Mol Mutagen* 2009 ; 50 : 733-40.

10. Fu J, Glover DM. Structured illumination of the interface between centriole and peri-centriolar material. *Open Biol* 2012 ; 2 : 120104-.

11. Lawo S, Hasegan M, Gupta GD, Pelletier L. Subdiffraction imaging of centrosomes reveals higher-order organizational features of pericentriolar material. *Nat Cell Biol* 2012 ; 14 : 1148-58.

12. Sonnen KF, Schermelleh L, Leonhardt H, Nigg EA. 3D-structured illumination microscopy provides novel insight into architecture of human centrosomes. *Biol Open* 2012 ; 1 : 965-76.

13. Okuda M, Horn HF, Tarapore P, *et al*. Nucleophosmin/B23 is a target of CDK2/cyclin E in centrosome duplication. *Cell* 2000 ; 103 : 127-40.

14. Ma Z, Kanai M, Kawamura K, Kaibuchi K, Ye K, Fukasawa K. Interaction between ROCK II and nucleophosmin/B23 in the regulation of centrosome duplication. *Mol Cell Biol* 2006 ; 26 : 9016-34.

15. Gönczy P, Hatzopoulos GN. Centriole assembly at a glance. *J Cell Sci* 2019 ; 132 : jcs228833.

16. Avidor-Reiss T, Gopalakrishnan J. Building a centriole. *Curr Opin Cell Biol* 2013 ; 25 : 72-7.

17. Li J, Kim S, Kobayashi T, *et al*. Neurl4, a novel daughter centriole protein, prevents formation of ectopic microtubule organizing centres. *EMBO Rep* 2012 ; 13 : 547-53.

18. Hori A, Toda T. Regulation of centriolar satellite integrity and its physiology. *Cell Mol Life Sci* 2017 ; 74 : 213-29.

19. Mitelman F, Johansson B, Mertens F, eds. *Mitelman database of chromosome aberrations and gene fusions in cancer*, 2018 [date unknown] http://cgap.nci.nih.gov/Chromosomes/Mitelman.

20. Boveri T. Concerning the origin of malignant tumours by Theodor Boveri. Translated and annotated by Henry Harris. *J Cell Sci* 2008 ; 121 : 1.

21. Zyss D, Gergely F. Centrosome function in cancer: guilty or innocent? *Trends Cell Biol* 2009 ; 19 : 334-46.

22. Levine MS, Bakker B, Boeckx B, *et al*. Centrosome amplification is sufficient to promote spontaneous tumorigenesis in mammals. *Dev Cell* 2017 ; 40 : 313-322.e5.

23. Marthiens V, Piel M, Basto R. Never tear us apart – the importance of centrosome clustering. *J Cell Sci* 2012 ; 125 : 3281-92.

24. Milunović-Jevtić A, Mooney P, Sulerud T, Bisht J, Gatlin J. Centrosomal clustering contributes to chromosomal instability and cancer. *Curr Opin Biotechnol* 2016 ; 40 : 113-8.

25. Cunha-Ferreira I, Rodrigues-Martins A, Bento I, *et al*. The SCF/Slimb ubiquitin ligase limits centrosome amplification through degradation of SAK/PLK4. *Curr Biol* 2009 ; 19 : 43-9.

26. Marthiens V, Rujano MA, Pennetier C, Tessier S, Paul-Gilloteaux P, Basto R. Centrosome amplification causes microcephaly. *Nat Cell Biol* 2013 ; 15 : 731-40.

27. Habedanck R, Stierhof Y-D, Wilkinson CJ, Nigg EA. The Polo kinase Plk4 functions in centriole duplication. *Nat Cell Biol* 2005 ; 7 : 1140-6.

28. Karki M, Keyhaninejad N, Shuster CB. Precocious centriole disengagement and centrosome fragmentation induced by mitotic delay. *Nat Commun* 2017 ; 8 : 15803.

29. Meraldi P, Honda R, Nigg AE. Aurora-A overexpression reveals tetraploidization as a major route to centrosome amplification in p53−/− cells. *EMBO J* 2002 ; 21 : 483-92.

30. Fukasawa K, Choi T, Kuriyama R, Rulong S, Vande Woude GF. Abnormal centrosome amplification in the absence of p53. *Science* 1996 ; 271 : 1744-7.

31. Tarapore P, Horn HF, Tokuyama Y, Fukasawa K. Direct regulation of the centrosome duplication cycle by the p53-p21$^{Waf1/Cip1}$ pathway. *Oncogene* 2001 ; 20 : 3173-84.

32. Cuomo ME, Knebel A, Morrice N, Paterson H, Cohen P, Mittnacht S. p53-driven apoptosis limits centrosome amplification and genomic instability downstream of NPM1 phosphorylation. *Nat Cell Biol* 2008 ; 10 : 723-30.

33. Holland AJ, Fachinetti D, Zhu Q, *et al*. The autoregulated instability of Polo-like kinase 4 limits centrosome duplication to once per cell cycle. *Genes Dev* 2012 ; 26 : 2684-9.

34. Fava LL, Schuler F, Sladky V, *et al*. The PIDDosome activates p53 in response to supernumerary centrosomes. *Genes Dev* 2017 ; 31 : 34-45.

35. Coelho PA, Bury L, Shahbazi MN, *et al*. Over-expression of Plk4 induces centrosome amplification, loss of primary cilia and associated tissue hyperplasia in the mouse. *Open Biol* 2015 ; 5 : 150209.

36. Serçin Ö, Larsimont J-C, Karambelas AE, *et al*. Transient PLK4 overexpression accelerates tumorigenesis in p53-deficient epidermis. *Nat Cell Biol* 2016 ; 18 : 100-10.

37. Duensing S, Lee LY, Duensing A, *et al*. The human papillomavirus type 16 E6 and E7 oncoproteins cooperate to induce mitotic defects and genomic instability by uncoupling centrosome duplication from the cell division cycle. *Proc Natl Acad Sci U S A* 2000 ; 97 : 10002-7.

38. Skyldberg B, Fujioka K, Hellström A-C, Sylvén L, Moberger B, Auer G. Human papillomavirus infection, centrosome aberration, and genetic stability in cervical lesions. *Mod Pathol* 2001 ; 14 : 279.

39. Duensing S, Duensing A, Lee DC, *et al*. Cyclin-dependent kinase inhibitor indirubin-3′-oxime selectively inhibits human papillomavirus type 16 E7-induced numerical centrosome anomalies. *Oncogene* 2004 ; 23 : 8206-15.

40. Duensing A, Liu Y, Tseng M, Malumbres M, Barbacid M, Duensing S. Cyclin-dependent kinase 2 is dispensable for normal centrosome duplication but required for oncogene-induced centrosome overduplication. *Oncogene* 2006 ; 25 : 2943.

41. Duensing A, Liu Y, Perdreau SA, Kleylein-Sohn J, Nigg EA, Duensing S. Centriole overduplication through the concurrent formation of multiple daughter centrioles at single maternal templates. *Oncogene* 2007 ; 26 : 6280-8.

42. Duensing A, Liu Y, Spardy N, *et al*. RNA polymerase II transcription is required for human papillomavirus type 16 E7- and hydroxyurea-induced centriole overduplication. *Oncogene* 2007 ; 26 : 215-23.

43. Korzeniewski N, Treat B, Duensing S. The HPV-16 E7 oncoprotein induces centriole multiplication through deregulation of Polo-like kinase 4 expression. *Mol Cancer* 2011 ; 10 : 61.

44. Kwun HJ, Wendzicki JA, Shuda Y, Moore PS, Chang Y. Merkel cell polyomavirus small T antigen induces genome instability by E3 ubiquitin ligase targeting. *Oncogene* 2017 ; 36 : 6784.

45. Martínez-Noël G, Luck K, Kühnle S, *et al*. Network analysis of UBE3A/E6AP-associated proteins provides connections to several distinct cellular processes. *J Mol Biol* 2018 ; 430 : 1024-50.

46. Nguyen CL, Eichwald C, Nibert ML, Munger K. Human papillomavirus type 16 E7 oncoprotein associates with the centrosomal component-tubulin. *J Virol* 2007 ; 81 : 13533-43.

47. Afonso PV, Zamborlini A, Saïb A, Mahieux R. Centrosome and retroviruses: the dangerous liaisons. *Retrovirology* 2007 ; 4 : 27.

48. Nitta T, Kanai M, Sugihara E, *et al*. Centrosome amplification in adult T-cell leukemia and human T-cell leukemia virus type 1 Tax-induced human T cells. *Cancer Sci* 2006 ; 97 : 836-41.

49. Peloponese J-M, Haller K, Miyazato A, Jeang K-T. Abnormal centrosome amplification in cells through the targeting of Ran-binding protein-1 by the human T cell leukemia virus type-1 Tax oncoprotein. *Proc Natl Acad Sci U S A* 2005 ; 102 : 18974-9.

50. Ching Y-P, Chan S-F, Jeang K-T, Jin D-Y. The retroviral oncoprotein Tax targets the coiled-coil centrosomal protein TAX1BP2 to induce centrosome overduplication. *Nat Cell Biol* 2006 ; 8 : 717-24.

51. Forgues M, Difilippantonio MJ, Linke SP, *et al*. Involvement of Crm1 in hepatitis B virus X protein-induced aberrant centriole replication and abnormal mitotic spindles. *Mol Cell Biol* 2003 ; 23 : 5282-92.

52. Yun C, Cho H, Kim S-J, *et al*. Mitotic aberration coupled with centrosome amplification is induced by hepatitis B virus X oncoprotein *via* the Ras-mitogen-activated protein/extracellular signal-regulated kinase-mitogen-activated protein pathway. *Mol Cancer Res MCR* 2004 ; 2 : 159-69.

53. Wang LH-C, Huang W, Lai M-D, Su I-J. Aberrant cyclin A expression and centrosome overduplication induced by hepatitis B virus Pre-S2 mutants and its implication in hepatocarcinogenesis. *Carcinogenesis* 2012 ; 33 : 466-72.

54. Jin D-Y, Spencer F, Jeang K-T. Human T cell leukemia virus type 1 oncoprotein Tax targets the human mitotic checkpoint protein MAD1. *Cell* 1998 ; 93 : 81-91.

55. Swanton C, Mann DJ, Fleckenstein B, Neipel F, Peters G, Jones N. Herpes viral cyclin/Cdk6 complexes evade inhibition by CDK inhibitor proteins. *Nature* 1997 ; 390 : 184-7.

56. Verschuren EW, Klefstrom J, Evan GI, Jones N. The oncogenic potential of Kaposi's sarcoma-associated herpesvirus cyclin is exposed by p53 loss *in vitro* and *in vivo*. *Cancer Cell* 2002 ; 2 : 229-41.

57. Verschuren EW, Hodgson JG, Gray JW, Kogan S, Jones N, Evan GI. The role of p53 in suppression of KSHV cyclin-induced lymphomagenesis. *Cancer Res* 2004 ; 64 : 581-9.

58. Koopal S, Furuhjelm JH, Järviluoma A, *et al*. Viral oncogene-induced DNA damage response is activated in kaposi sarcoma tumorigenesis. *PLoS Pathog* 2007 ; 3 : e140.

59. Pan H, Zhou F, Gao S-J. Kaposi's sarcoma-associated herpesvirus induction of chromosome instability in primary human endothelial cells. *Cancer Res* 2004 ; 64 : 4064-8.

60. Lane DP, Crawford LV. T antigen is bound to a host protein in SV40-transformed cells. *Nature* 1979 ; 278 : 261-3.

61. Linzer DIH, Levine AJ. Characterization of a 54K Dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. *Cell* 1979 ; 17 : 43-52.

62. Tornesello ML, Annunziata C, Tornesello AL, Buonaguro L, Buonaguro FM. Human oncoviruses and p53 tumor suppressor pathway deregulation at the origin of human cancers. *Cancers (Basel)* 2018 ; 10 : 213.

63. Riley RR, Duensing S, Brake T, Münger K, Lambert PF, Arbeit JM. Dissection of human papillomavirus E6 and E7 function in transgenic mouse models of cervical carcinogenesis. *Cancer Res* 2003 ; 63 : 4862-71.

64. Budhu AS, Wang XW. Loading and unloading. *Cell Cycle* 2005 ; 4 : 1510-4.

65. Keryer G, Di Fiore B, Celati C, *et al*. Part of Ran is associated with AKAP450 at the centrosome: involvement in microtubule-organizing activity. *Mol Biol Cell* 2003 ; 14 : 4260-71.

66. Ciciarello M, Mangiacasale R, Lavia P. Spatial control of mitosis by the GTPase Ran. *Cell Mol Life Sci* 2007 ; 64 : 1891-914.

67. Di Fiore B, Ciciarello M, Mangiacasale R, *et al*. Mammalian RanBP1 regulates centrosome cohesion during mitosis. *J Cell Sci* 2003 ; 116 : 3399-411.

68. Luca AD, Mangiacasale R, Severino A, *et al*. E1A deregulates the centrosome cycle in a Ran GTPase-dependent manner. *Cancer Res* 2003 ; 63 : 1430-7.

69. Lavia P. The GTPase RAN regulates multiple steps of the centrosome life cycle. *Chromosome Res* 2016 ; 24 : 53-65.

70. Gill MB, Kutok JL, Fingeroth JD. Epstein-Barr virus thymidine kinase is a centrosomal resident precisely localized to the periphery of centrioles. *J Virol* 2007 ; 81 : 6523-35.

71. Shumilov A, Tsai M-H, Schlosser YT, *et al*. Epstein-Barr virus particles induce centrosome amplification and chromosomal instability. *Nat Commun* 2017 ; 8 : 14257.

72. Tsai K, Chan L, Gibeault R, *et al*. Viral reprogramming of the Daxx histone H3.3 chaperone during early Epstein-Barr virus infection. *J Virol* 2014 ; 88 : 14350-63.

73. Baek K-H, Park H-Y, Kang C-M, *et al*. Overexpression of hepatitis C virus NS5A protein induces chromosome instability *via* mitotic cell cycle dysregulation. *J Mol Biol* 2006 ; 359 : 22-34.

74. Kwon M, Godinho SA, Chandhok NS, *et al*. Mechanisms to suppress multipolar divisions in cancer cells with extra centrosomes. *Genes Dev* 2008 ; 22 : 2189-203.

75. Leber B, Maier B, Fuchs F, *et al*. Proteins required for centrosome clustering in cancer cells. *Sci Transl Med* 2010 ; 2 : 33ra38-.

76. Quintyne NJ, Reing JE, Hoffelder DR, Gollin SM, Saunders WS. Spindle multipolarity is prevented by centrosomal clustering. *Science* 2005 ; 307 : 127-9.

77. Kwon M, Godinho SA, Chandhok NS, *et al*. Mechanisms to suppress multipolar divisions in cancer cells with extra centrosomes. *Genes Dev* 2008 ; 22 : 2189-203.

78. Ying B, Wold WSM. Adenovirus ADP protein (E3-11.6K), which is required for efficient cell lysis and virus release, interacts with human MAD2B. *Virology* 2003 ; 313 : 224-34.

79. Cotsiki M, Lock RL, Cheng Y, *et al*. Simian virus 40 large T antigen targets the spindle assembly checkpoint protein Bub1. *Proc Natl Acad Sci U S A* 2004 ; 101 : 947-52.

80. Patel D, McCance DJ. Compromised spindle assembly checkpoint due to altered expression of Ubch10 and Cdc20 in human papillomavirus type 16 E6- and E7-expressing keratinocytes. *J Virol* 2010 ; 84 : 10956-64.

81. Chae S, Ji J-H, Kwon S-H, *et al*. HBxAPα/Rsf-1-mediated HBx-hBubR1 interactions regulate the mitotic spindle checkpoint and chromosome instability. *Carcinogenesis* 2013 ; 34 : 1680-8.

82. Tan CL, Teissier S, Gunaratne J, Quek LS, Bellanger S. Stranglehold on the spindle assembly checkpoint: the human papillomavirus E2 protein provokes BUBR1-dependent aneuploidy. *Cell Cycle* 2015 ; 14 : 1459-70.

83. Shirnekhi HK, Kelley EP, DeLuca JG, Herman JA. Spindle assembly checkpoint signaling and sister chromatid cohesion are disrupted by HPV E6-mediated transformation. *Mol Biol Cell* 2017 ; 28 : 2035-41.

84. Arquint C, Gabryjonczyk A-M, Nigg EA. Centrosomes as signalling centres. *Philos Trans R Soc Lond B Biol Sci* 2014 ; 369 : 20130464-.

85. Löffler H, Fechter A, Matuszewska M, *et al*. Cep63 Recruits Cdk1 to the centrosome: implications for regulation of mitotic entry, centrosome amplification, and genome maintenance. *Cancer Res* 2011 ; 71 : 2129-39.

86. Dutertre S. Phosphorylation of CDC25B by Aurora-A at the centrosome contributes to the G2-M transition. *J Cell Sci* 2004 ; 117 : 2523-31.

87. Ciccia A, Elledge SJ. The DNA damage response: making it safe to play with knives. *Mol Cell* 2010 ; 40 : 179-204.

88. Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* 2003 ; 421 : 499.

89. Zhang S, Hemmerich P, Grosse F. Centrosomal localization of DNA damage checkpoint proteins. *J Cell Biochem* 2007 ; 101 : 451-65.

90. Oricchio E, Saladino C, Iacovelli S, Soddu S, Cundari E. ATM is activated by default in mitosis, localizes at centrosomes and monitors mitotic spindle integrity. *Cell Cycle* 2006 ; 5 : 88-92.

91. Wang C-Y, Huang EY-H, Huang S-C, Chung B-C. DNA-PK/Chk2 induces centrosome amplification during prolonged replication stress. *Oncogene* 2015 ; 34 : 1263-9.

92. Löffler H, Bochtler T, Fritz B, *et al*. DNA damage-induced accumulation of centrosomal Chk1 contributes to its checkpoint function. *Cell Cycle* 2007 ; 6 : 2541-8.

93. Dutton A, Woodman CB, Chukwuma MB, *et al*. Bmi-1 is induced by the Epstein-Barr virus oncogene LMP1 and regulates the expression of viral target genes in Hodgkin lymphoma cells. *Blood* 2007 ; 109 : 2597-603.

94. Dayaram T, Lemoine FJ, Donehower LA, Marriott SJ. Activation of WIP1 phosphatase by HTLV-1 Tax mitigates the cellular response to DNA damage. *PLoS One* 2013 ; 8 : e55989.

95. Choudhuri T, Verma SC, Lan K, Murakami M, Robertson ES. The ATM/ATR signaling effector Chk2 is targeted by Epstein-Barr virus nuclear antigen 3C to release the G2/M cell cycle block. *J Virol* 2007 ; 81 : 6718-30.

96. Gupta SK, Guo X, Durkin SS, Fryrear KF, Ward MD, Semmes OJ. Human T-cell leukemia virus type 1 tax oncoprotein prevents DNA damage-induced chromatin egress of hyperphosphorylated Chk2. *J Biol Chem* 2007 ; 282 : 29431-40.

97. Robinson HMR, Black EJ, Brown R, Gillespie DAF. DNA mismatch repair and Chk1-dependent centrosome amplification in response to DNA alkylation damage. *Cell Cycle* 2007 ; 6 : 982-92.

98. Antonczak AK, Mullee LI, Wang Y, *et al*. Opposing effects of pericentrin and microcephalin on the pericentriolar material regulate CHK1 activation in the DNA damage response. *Oncogene* 2016 ; 35 : 2003.

99. Inanç B, Dodson H, Morrison CG. A centrosome-autonomous signal that involves centriole disengagement permits centrosome duplication in G2 phase after DNA damage. *Mol Biol Cell* 2010 ; 21 : 3866-77.

100. Conroy PC, Saladino C, Dantas TJ, Lalor P, Dockery P, Morrison CG. C-NAP1 and rootletin restrain DNA damage-induced centriole splitting and facilitate ciliogenesis. *Cell Cycle* 2012 ; 11 : 3769-78.

101. Lindgren T, Stigbrand T, Johansson L, Riklund K, Eriksson D. Alterations in gene expression during radiation-induced mitotic catastrophe in HeLa Hep2 cells. *Anticancer Res* 2014 ; 34 : 3875-80.

102. Mussman JG, Horn HF, Carroll PE, *et al*. Synergistic induction of centrosome hyperamplification by loss of p53 and cyclin E overexpression. *Oncogene* 2000 ; 19 : 1635-46.

103. Shinmura K, Bennett RA, Tarapore P, Fukasawa K. Direct evidence for the role of centrosomally localized p53 in the regulation of centrosome duplication. *Oncogene* 2007 ; 26 : 2939-44.

104. Löffler H, Fechter A, Liu FY, Poppelreuther S, Krämer A. DNA damage-induced centrosome amplification occurs *via* excessive formation of centriolar satellites. *Oncogene* 2013 ; 32 : 2963-72.

105. Smith E, Dejsuphong D, Balestrini A, *et al*. An ATM- and ATR-dependent checkpoint inactivates spindle assembly by targeting CEP63. *Nat Cell Biol* 2009 ; 11 : 278-85.

106. Lai WL, Hung WY, Ching YP. The tumor suppressor, TAX1BP2, is a novel substrate of ATM kinase. *Oncogene* 2014 ; 33 : 5303-9.

107. Wang M, Knudsen BS, Nagle RB, Rogers GC, Cress AE. A method of quantifying centrosomes at the single-cell level in human normal and cancer tissue. *Mol Biol Cell* 2019 ; 30 : 811-9.

108. Firat-Karalar EN, Rauniyar N, Yates JR, Stearns T. Proximity interactions among centrosome components identify regulators of centriole duplication. *Curr Biol* 2014 ; 24 : 664-70.