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Viral transformation and aneuploidy

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Abstract

Human tumor viruses are associated with a variety of human malignancies, and it is estimated that 15% of all human cancers have a viral etiology. An abnormality in chromosomal ploidy or aneuploidy is a hallmark of cancers. In normal cells, euploidy is governed by several factors including an intact spindle assembly checkpoint (SAC), accurate centrosome duplication, and proper cytokinesis. Viral oncoproteins are suggested to perturb the cellular machineries for chromosomal segregation creating aneuploidy which can lead to the malignant transformation of infected cells. Here we review in brief some of the mechanisms employed by viruses that can cause cellular aneuploidy.

Keywords

Spindle assembly checkpoint; centrosome; aneuploidy; human T-cell leukemia virus type I (HTLV-I); Tax

Introduction

Viruses are one of the major etiologies of human cancers. There are six major human cancer viruses. These include human T-cell leukemia virus type I (HTLV-I), hepatitis B virus (HBV), hepatitis C virus (HCV), human papillomavirus (HPV), Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV). HTLV-I is a retrovirus that causes adult T-cell leukemia (ATL) (Matsuoka and Jeang, 2007). HBV and HCV cause hepatocellular carcinoma (HCC) (Bruix et al., 2006). HPVs induce cervical cancer (Woodman et al., 2007). EBV is associated with Burkitt's lymphoma, and nasopharyngeal carcinoma (NPC) (Pattle and Farrell, 2006) amongst others; KSHV is causal for Kaposi's sarcoma in the compromised hosts (Cathomas, 2000).

The number of virus-related malignancies is estimated to encompass 15% of all human cancers worldwide (zur, 1991). Achievements in the research on viral oncogenic mechanisms have contributed to a better understanding of various biological events in the cell. Indeed, emerging discoveries of new viral causes of cancers such as the human xenotropic murine leukemia virus (MLV)-related virus (XMRV) in human prostate cancer (Urisman et al., 2006) and the human MC-polyomavirus in Merkel cell carcinoma (Feng et al., 2008) raise the hope of elucidating viral explanations for the genesis of many heretofore unexplained cancers.

Mechanisms of aneuploidy

Aneuploidy is a common feature of many cancers (Chi and Jeang, 2007). A normal mammalian somatic cell is diploid. Thus, a human nucleus has 46 chromosomes, which are precisely duplicated and segregated in mitosis with fidelity to two daughter nuclei. An improper loss or gain in chromosomes can occur through the four events described below (Figure 1). Other means for creating aneuploidy are also possible.

a) The aberrant attachment of spindle microtubules to duplicated chromatids coupled with a defect in the spindle assembly checkpoint (SAC)

In a normal mitosis, duplicated chromosomes pair and congress at the metaphase plate during metaphase and are attached via spindle microtubules to bipolar spindle poles. Each chromosome pair is tethered to the two poles with equal tension, and this bilateral attachment results in unbiased segregation of DNA into two daughter nuclei. When an improper attachment of spindle microtubules to the chromatids occurs (such as in a merotelic attachment), this error will produce missegregation. Spontaneous missegregations are censored by the SAC which monitors the correct attachment of the spindle microtubules to kinetochores (Cleveland et al., 2003). The protein constituents of the SAC include the Mad1, Mad2, Mad3/BubR1 (designated Mad3 in yeast and BubR1 in other species), Bub1, Bub3, and Mps1 proteins. A loss of SAC function and the mutation of some of the genes that encode for SAC proteins have been reported in cancer cells (Malmanche et al., 2006). In animal models, knocking out spindle checkpoint proteins in mice has been found to increase cellular aneuploidy and the incidence of *in vivo* tumor development (Dai et al., 2004; Iwanaga et al., 2007; Kalitsis et al., 2000; Michel et al., 2001); (Chi et al., 2008b); (Schliekelman et al., 2009).

b) Abnormal breakage-fusion-bridge (BFB)

The breakage-fusion-bridge (BFB) is a well-established mechanism for gene amplification. The BFB cycle is initiated when a chromosome undergoes a double-stranded break, replicates, and the sister chromatids fuse at their ends. Due to the presence of two centromeres, the fused sister chromatids are drawn towards both poles and form a bridge during anaphase. During cytokinesis, these dicentric chromosomes are broken unevenly and may fuse again during interphase, resulting in the next BFB cycle. By this type of cycling, the number chromosomes could become abnormally increased or decreased (Murnane, 2006; Fenech, 2006). The rearrangement of chromosomes through BFB cycles may be another possible source of aneuploidy (Gisselsson et al., 2000; Dey, 2004).

c) Failed cytokinesis

In the cell cycle, cytokinesis follows mitosis. While mitosis leads to the production of two daughter nuclei, cytokinesis divides a parent cell into two daughter cells such that each daughter receives a single nucleus. If cytokinesis does not occur, then the undivided cell becomes binucleated and tetraploid (Gisselsson, 2003). Several studies have shown how tetraploid cells can lead to aneuploid progenies in subsequent cell cycles (Fujiwara et al., 2005; Shi and King, 2005).

d) Aberrant duplication of centrosomes

During the cell cycle, a single centrosome is duplicated once in interphase. Each of the two duplicated centrosomes then becomes one of the two bipolar spindle poles during mitosis. When errors in duplication occur to produce more than two centrosomes (supernumerary centrosomes), a multipolar mitosis ensues. Multipolar mitosis with more than two spindle poles invariably results in aneuploidy. Several cellular proteins, including p53, BRCA1,

CHK1, CHK2, Ran GTPase, Aurora A, PLK1, Cyclin B1 and CDK1, regulate centrosome duplication and its function (Chi and Jeang, 2007;Kramer et al., 2004). Not surprisingly, aberrant centrosome numbers are common in many types of cancers including breast, lung, bone, pancreas, colorectal, prostate, head, and neck (Saunders, 2005).

An example of virus-cell transformation: HTLV-I and adult T-cell leukemia (ATL)

HTLV-I is the etiological agent of adult T cell leukemia (ATL), a malignancy of the peripheral CD4+ T lymphocytes. ATL cells are morphologically characterized by hyper-lobulated nuclei and are called “flower cells” (Matsuoka and Jeang, 2007) (Figure 2). The reason for the hyper-lobulated nuclei appears to be because most ATL cells have severe chromosomal aberrations including aneuploidy (Itoyama et al., 2001;Tsukasaki et al., 2001). The development of aneuploidy is likely one step in a multistep leukemogenic process in the development of ATL (Yasunaga and Matsuoka, 2003; Grassmann et al., 2005).

While the precise mechanisms of ATL leukemogenesis have not been fully clarified, it is thought that the HTLV-I encoded Tax oncoprotein plays a central role in cellular transformation (Boxus et al., 2008; Grassmann, Aboud, and Jeang, 2005). Tax can potentially activate the transcription of numerous cellular genes, and recent findings suggest that it can also change the expression profile of putatively oncogenic cellular microRNAs (Yeung et al., 2008; Bouzar and Willems, 2008; Pichler et al., 2008; Grassmann and Jeang, 2008). Tax can functionally inactivate many cellular proteins through direct interaction (Boxus, Twizere, Legros, Dewulf, Kettmann, and Willems, 2008; Ramadan et al., 2008). Importantly, expression of Tax-alone can transform rodent cells (Tanaka et al., 1990), immortalize human cord blood and peripheral blood cells (Akagi and Shimotohno, 1993; Grassmann et al., 1989; Grassmann et al., 1992; Rosin et al., 1998), and create tumors in Tax-transgenic mice (Green et al., 1992; Grossman et al., 1995; Hasegawa et al., 2006; Hinrichs et al., 1987).

Above, we noted that aneuploidy is frequently associated with cellular transformation. In studies of HTLV-I biology, it has been shown that the viral Tax oncoprotein can interact with cellular proteins involved in the SAC and in the regulation of centrosome duplication (Figure 2) (Afonso et al., 2007;Merling et al., 2007;Pumfery et al., 2006). Indeed, the hSMAD1 protein (Chi et al., 2008a) is a SAC component which is targeted by Tax for functional inactivation (Haller et al., 2006;Jin et al., 1998). In addition, Tax dysregulates the replication of centrosomes by associating with several cellular factors (Afonso, Zamborlini, Saib, and Mahieux, 2007). Moreover, Tax can interact with RanBP1, resulting in the fragmentation of centrosomes (Peloponese, Jr. et al., 2005), and Tax can bind TAX1BP2 to create the over-amplification of centrosomes (Ching et al., 2006). Furthermore, consistent with these experimental findings, it has been reported that the peripheral blood lymphocytes isolated from ATL patients frequently have an abnormal number of centrosomes (Nitta et al., 2006). Also, it has been shown that the incidence of multinucleated cells is significantly higher in HTLV-I- infected CD4+ T cell clones than in uninfected cells, and this finding is correlated with the expression level of *tax* mRNA. Since the T cell clones that were used in the reported study were not transformed, the results suggest that aneuploidy induced by Tax occurs prior to cellular transformation (Sibon et al., 2006).

The creation of chromosomal aberrations is censored unless the checkpoints that guard against their manifestation are also inactivated (Kasai et al., 2002). In this regard, Tax inhibits the functions of checkpoint proteins, such as p53 (Reid et al., 1993), pRB (Neuveut et al., 1998), CHK1 (Park et al., 2004), CHK2 (Haoudi et al., 2003) and others (Matsuoka and Jeang, 2007). Thus by creating DNA errors and also attenuating the cellular checks

against these mistakes, HTLV-I creates chromosomal instability and aneuploidy, thereby permitting the emergence of downstream cellular transformation events.

Other examples of virus-cell transformation

a) Human papillomavirus (HPV)

Infection with high-risk HPVs, such as HPV-16 and HPV-18, is associated with approximately 70% of cervical and anogenital cancers worldwide (Carrillo-Infante et al., 2007). High-risk HPVs encode two oncoproteins, E6 and E7, and both contribute to HPV-induced cellular transformation. E6 interacts with p53 and promotes its degradation (Scheffner et al., 1993), whereas E7 binds and inactivates the retinoblastoma tumor suppressor gene (pRB) (Dyson et al., 1989) (Table 1). It has been reported that HPV16 E7 rapidly induces an excessive production of centrioles (Duensing et al., 2007; Duensing et al., 2000), and E6 was found to provoke an accumulation of centrosomes (Duensing et al., 2008). Thus, both HPV-16 E6 and E7 can create abnormalities in centrosome numbers which can lead to aneuploid mitoses.

b) Hepatitis B (HBV) and hepatitis C (HCV) virus

More than 85% of HCC cases are associated with HBV or HCV (Forgues et al., 2003). HBV is a DNA virus that encodes the X oncoprotein (HBx). The *HBx* gene is frequently integrated into the host genome and is expressed during the development of HCC. Since the HBx protein can transform cultured cells (Hohne et al., 1990; Koike et al., 1994) and induce liver cancers in transgenic mice (Kim et al., 1991; Yu et al., 1999), this protein is thought to be critical in virus induced liver carcinogenesis. A cellular factor Crm1 is reported to play a role in maintaining the fidelity of centrosome duplication. HBx has been reported to interact with Crm1 and affect its localization (Table 1). A current notion is that HBx induces supernumerary centrosomes, multipolar spindles, and consequently aneuploidy via a disturbance of Crm1 function (Forgues et al., 2001; Forgues, Difilippantonio, Linke, Ried, Nagashima, Feden, Valerie, Fukasawa, and Wang, 2003). On the other hand, it has also been reported that HBx induces centrosome amplification and mitotic aberration through activation of the Ras-MEK-MAPK pathway (Yun et al., 2004).

HCV is a member of the positive strand RNA viruses. An HCV nonstructural protein, NS5A, is a phosphoprotein which interacts with cellular proteins such as PKR (Gale, Jr. et al., 1997), Cdk1 (Arima et al., 2001) and others. It has been shown that the over expression of NS5A dysregulates the cell cycle and creates aneuploidy in cultured liver cells (Baek et al., 2006). Recently, it was found that NS5A down-regulates the transcription of the mitotic spindle protein *Aspm* via the PKR-p38 signaling pathway and induces an aberrant mitotic cell cycle (Wu et al., 2008) (Table 1).

c) Kaposi sarcoma-associated herpesvirus (KSHV)

KSHV is a herpesvirus that is associated with Kaposi's sarcoma, primary effusion lymphoma, and Castleman's disease. KSHV encodes approximately 90 genes in its genome. Among them, KSHV-encoded cyclin D homolog, K cyclin, is considered to promote viral oncogenesis. It has been reported that K cyclin transgenic mice (*Eμ-K cyclin*) develop lymphoma, and a loss of p53 (*Eμ-K cyclin/p53^{-/-}*) function increases K cyclin-triggered tumor incidence (Verschuren et al., 2004) while enhancing centrosome abnormalities in mouse fibroblasts (Verschuren et al., 2002). Phosphorylation of nucleophosmin (NPM1) was recently identified as a molecular mechanism for centrosome amplification by K cyclin (Cuomo et al., 2008) (Table 1). Moreover, K cyclin activates CDK6, which phosphorylates NPM1. Hence, it is thought that K cyclin-induced NPM1 phosphorylation triggers centrosome amplification. Additionally, two other KSHV-encoded latent proteins, LANA

and vFLIP, augment significant accumulation of supernumerary centrosomes in K cyclin expressing cells. Collectively, the evidence shows that KSHV has evolved several means to induce aneuploidy.

d) Epstein-Barr virus (EBV)

EBV is considered to be causal of malignant diseases, such as Burkitt's lymphoma, nasopharyngeal carcinoma, and some Hodgkin's disease. EBV's latent membrane protein 1 (LMP1) has been reported to transform rodent fibroblasts (Wang et al., 1985) and activate the NF- κ B signaling pathways. A tumor suppressor gene *RASSF1A* is known to stabilize cellular microtubules and regulate mitotic events, and its inactivation induces abnormal spindle formation and chromosomal instability (Liu et al., 2003; Rong et al., 2004). EBV LMP1 has been found to down-regulate the expression of *RASSF1A* through an NF- κ B pathway, resulting in aberrant mitotic spindles and aneuploid chromosomes (Man et al., 2007) (Table 1). In addition, latent EBV infection impairs the mitotic SAC and rescues Burkitt's lymphoma-derived cells from other caspase-dependent cell death when cultured *in vitro* (Leao et al., 2007).

Concluding remarks

It is still debatable whether aneuploidy is the cause or the consequence of cellular transformation. As described here in brief, there is evidence that some human cancer viruses encode viral oncoproteins that cause cellular aneuploidy. We note with interest that in similarity with human cancer viruses, bacterial *Helicobacter pylori* infection and its expression of bacterial CagA protein are also reportedly associated with the accumulation of chromosomal aberrations (Williams et al., 2005). Taken together, the findings converge to suggest that infection with many oncogenic pathogens can induce aneuploidy, and that aneuploidy may be the initial trigger for the development of cellular transformation.

To date, the research on viral oncogenesis has clarified many pathogenic etiologies and shed light on several critical cellular regulatory functions. A direct causality between infection, aneuploidy, and cancer remains to be conclusively established. However, increasingly, findings are consistent with these associations. Future research is needed to focus on clarifying better these linkages and their implications for novel therapeutic strategies against viral malignancies.

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Figure 1.

Pathways to aneuploidy. Aneuploidy can be caused by a) improper microtubule attachment to kinetochores with a defect in the SAC; b) abnormal BFB events; c) failure by a cell after mitosis to undergo proper cytokinesis; and d) aberrant amplification of supernumerary centrosomes with multipolar mitosis. Other mechanisms not illustrated here are also possible for the genesis of aneuploidy.

Figure 2.

An illustration of HTLV-I Tax-induced aneuploidy. Tax interacts with the spindle assembly checkpoint (SAC) protein, hSMAD1, and inhibits its function. Impairment of SAC permits cells to manifest spontaneous occurrence of unbalanced segregations of chromosomes in mitosis. Separately, Tax can bind RanBP1 and Tax1BP2 which regulate centrosome functions. Tax-induced loss of RanBP1/Tax1BP2 function creates supernumerary centrosomes and multipolar mitotic spindles. A putative result of Tax induced aneuploidy is the presentation of multi-lobulated nuclei in ATL cells, also called “flower cells”.

Table 1

Viral proteins associated with aneuploidy

Virus	Malignant diseases	Viral oncoprotein	Induced aberration in mitotic cells	Associated cellular factors
HTLV-I	ATL	Tax	Impairment of SAC	HsMAD1
			Centrosome fragmentation	RanBP1
			Centrosome amplification	Tax1BP2
HPV	Cervical cancer	E6	Centrosome accumulation	p53
	Anogenital cancer			
HBV	HCC	E7	Centrosome amplification	pRb
		HBx	Centriole amplification	Crm1
			Centrosome amplification	Ras-MEK-MAPK pathway
HCV	HCC	NS5A	Centrosome amplification	PKR
EBV	Burkitt's lymphoma	LMP1	Aberrant mitotic spindles	ASPM
	Nasopharyngeal carcinoma			RASSF1A
	Hodgkin's disease			
KSHV	Kaposi's sarcoma	K cyclin	Centrosome amplification	CDK6
				NPM1