Centromere Protein A Goes Far Beyond the Centromere in Cancers

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ABSTRACT

Centromere dysfunctions leading to numerical chromosome alterations are believed to be closely related to human cancers. As a centromere-specific protein, centromere protein A (CENP-A) replaces the histone H3 in centromeres and is therefore considered a key factor of centromere identity. Researches have shown that CENP-A is overexpressed in many types of human cancers. However, the behavior and function of CENP-A in tumorigenesis have not yet been systematically summarized. In this article, we describe the pleiotropic roles of CENP-A in human cells. Moreover, we provide a comprehensive review of the current knowledge on the relationship between aberrant expression and ectopic localization of CENP-A and tumorigenesis, and the mechanism of the ectopic deposition of CENP-A in cancers. Furthermore, we note that some oncogenic viruses can modulate the expression and localization of this centromere protein along with its chaperone. At last, we also discuss the therapeutic potential of targeting CENP-A for cancer therapy.

Introduction

The centromere is a specialized region on the chromosome that is essential for the faithful segregation of chromosomes during mitosis to ensure that each daughter cell receives one copy of the genome (1). Accurate centromeric assembly is vital for maintaining genomic stability, while centromere malfunction can lead to changes in chromosome numbers (2). Such chromosome abnormalities (aneuploidies) are near-universal characteristics of human cancers. However, centromere remains a largely unexplored area in cancer research (3). Detailed insights into the molecular mechanisms of centromere dysregulation and the function of centromere proteins will help to improve our understanding of cancer and to find a new way to fight it.

In humans, the chromosome centromeres are comprised of tandemly repeated arrays of a 171-bp DNA sequence element called α-satellite, but these repetitive elements are neither sufficient nor essential for centromere identity (4). Instead, centromeres are specified epigenetically by the presence of nucleosomes containing the centromere protein A (CENP-A). CENP-A is a 17-kD centromere-specific variant of histone H3 that is required for the assembly of centromere/kinetochore components and accurate chromosome segregation (5). The *CENP-A* gene locates at human chromosome 2p23.3, consists of 1389 bp with 5 coding exons, and encodes a protein of 140 amino acids. Its protein expression and assembly occur through a very tightly regulated process in the context of the cell cycle (6). As a key factor of centromere identity, CENP-A is found predominantly at the centromere by approximately 40-fold compared with other regions in the chromosome, which distinguishes the centromere from the rest of the chromatin (2). Although various compositions of CENP-A nucleosomes have been suggested, the octamer is the major form of CENP-A nucleosomes at human centromeres (7). Along with histones H2A, H2B, and H4, CENP-A assembles into centromeric nucleosomes and its centromeric localization is mediated by the histone chaperone HJURP (7, 8). CENP-A governs the centromere function by recruiting the constitutive centromere-associated network (CCAN) complex composed of 16 protein subunits. Two other members of the centromere protein family, CENP-C and CENP-N are the only two subunits of the CCAN complex that directly interacts with CENP-A nucleosomes (9). At present, the function of CENP-A in epigenetic regulation of centromere identity, propagation, and kinetochore assembly has been extensively reviewed by several investigators (6, 10, 11). With the development of research on centromere proteins over the past several decades, it has been found that CENP-A is overexpressed in a variety of human cancers (12–14). However, the behavior and function of CENP-A in tumorigenesis have not yet been comprehensively summarized and remain a challenge. In this paper, we review the recent advances and discuss our understanding of the relationship between aberrant expression and ectopic localization of CENP-A and tumorigenesis, as well as its potential roles in cancer therapeutics.

The regulatory mechanism and pleiotropic function of CENP-A

Centromere identity and function is a complex and tightly regulated process (15). As the epigenetic mark of centromere identity, the regulation of CENP-A expression involves transcriptional, posttranscriptional, and posttranslational processes (**Fig. 1**). Three genes (FOXM1, E2F8, and E2F2) that play critical function in tumorigenesis and cell-cycle regulation were identified as potential transcriptional regulators of *CENP-A* (16). Among them, FOXM1 has already been proven to interact directly with the promoter of *CENP-A*–based on chromatin immunoprecipitation sequencing (ChIP-seq). Besides, recent studies have also demonstrated that the tumor suppressor p53 can negatively regulate CENP-A at the transcript level (17, 18). In line with CENP-A overexpression, FOXM1, E2F2, and E2F8 are overexpressed in multiple human cancers (19–21), while the negative regulator p53 is often mutated. The promoter of *CENP-A* itself was also bound by CENP-A protein, which indicates that CENP-A might regulate its transcription (12). Interestingly, although the CENP-A mRNA levels in human pluripotent stem cells (hPSC) were significantly higher than somatic cells, the protein expression of CENP-A in hPSCs was identical to somatic cells (22). This discrepancy between the

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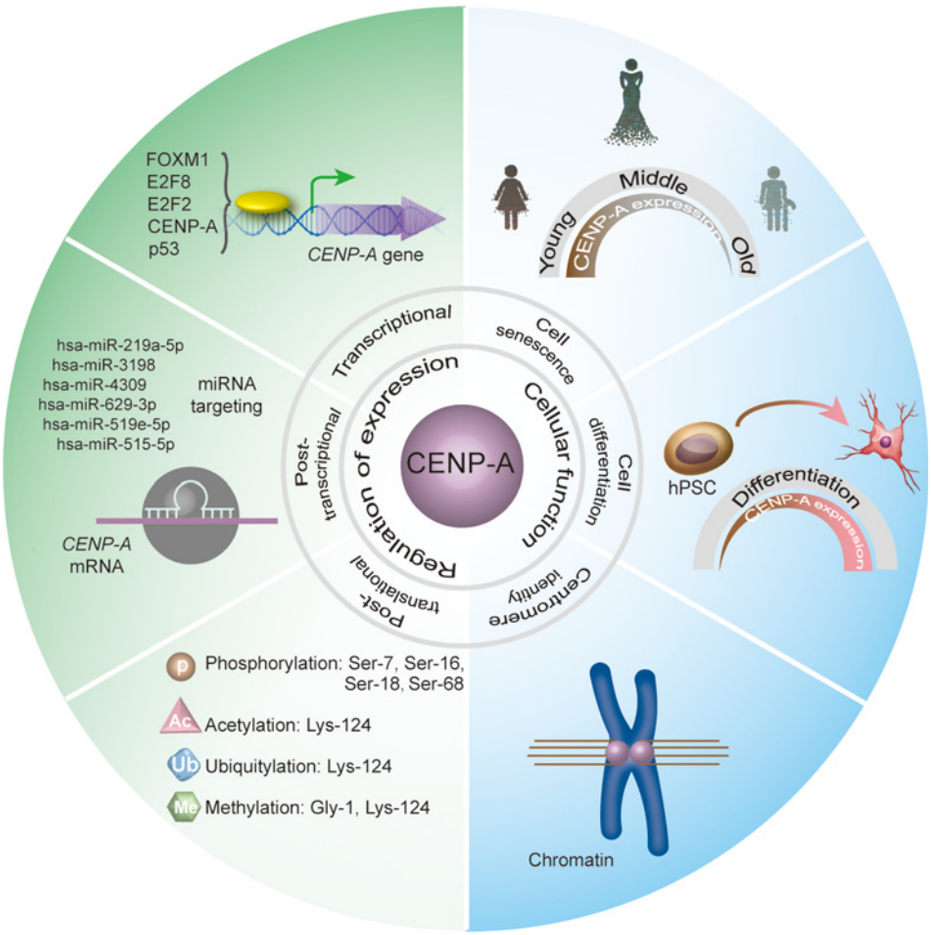

Figure 1.
An overview of CENP-A regulation and cellular function. As shown in the left semicircle, the expression of human CENP-A is regulated by different mechanisms, from transcriptional, posttranscriptional to posttranslational regulation. First, the cell-cycle transcription factors FOXM1, E2F8, E2F2, and the tumor-suppressor p53 as well as CENP-A itself were identified as the transcriptional regulators of *CENP-A* gene. In addition, six microRNAs (hsa-miR-219a-5p, hsa-miR-3198, hsa-miR-4309, hsa-miR-629–3p, hsa-miR-519e-5p and hsa-miR-515–5p) were identified as potential posttranscriptional regulators of CENP-A. In line with CENP-A overexpression, most of these microRNAs were downregulated in cancers. CENP-A is also subjected to posttranslational modifications including phosphorylation (Ser-7, Ser-16, Ser-18, and Ser-68), acetylation (Lys-124), methylation (Gly-1 and Lys-124), and ubiquitylation (Lys-124). The right semicircle shows the cellular function of CENP-A, including cell senescence, differentiation, and centromere identity. In humans, CENP-A is highly enriched at centromeres and serves as an epigenetic marker for centromere identity. Moreover, the expression levels of CENP-A were also reported to be increased in the process of differentiation while decreased in the process of senescence.

abundance of CENP-A protein and RNA in hPSCs could be explained by the unique RNA translational controls identified in murine pluripotent stem cells (PSC; ref. 23). In addition, six microRNAs were identified as potential posttranscriptional regulators of *CENP-A*, and most of these microRNAs were downregulated in cancers (16). Human CENP-A is also subjected to dynamic and versatile posttranslational modifications including phosphorylation (Ser-7, Ser-16, Ser-18, and Ser-68), acetylation (Lys-124), methylation (Gly-1 and Lys-124), and ubiquitylation (Lys-124), as discussed in detail in a recent review (24). Among these modification sites, phosphorylation of Ser-18 (25) and Ser-68 (26), ubiquitylation of Lys-124 (27) have been proven to affect CENP-A centromeric localization, while phosphorylation of Ser-7 (28), acetylation of Lys-124 (29), trimethylation of Gly-1 (30) appear to have an impact on the recruitment of CCAN-associated proteins to the centromere. It's worth noting that the importance of Ser-7 phosphorylation in centromere function has been questioned by a recent study demonstrating that this phosphorylation site is neither essential nor required for centromere function (31). Despite this, post-translational modification (PTM) still play an important role in CENP-A deposition at centromeres and CCAN recruitment. Besides, CENP-A protein is also regulated by proteolytic cleavage in multiple organisms such as *yeast species* and *Drosophila* (32, 33). Although human CENP-A is subjected to ubiquitin-proteasomal degradation, the identity of the corresponding E3 ubiquitin ligase remains unknown except for the herpes simplex virus type 1 (HSV-1)–encoded infected-cell protein 0 (ICP0; refs. 34, 35). During HSV-1 infection, the RING-finger E3 ubiquitin ligase ICP0 induces the proteasome-dependent degradation of CENP-A protein, leading to the loss of CENP-A from centromeres and cell-cycle arrest. The hijacking of host-cell ubiquitination machinery by HSV-1 might create an environment permissive for virus replication (36). However, this kind of E3 ligase encoded by the human genome has hitherto not been reported.

In addition to its essential role for centromere function, CENP-A is also found to be involved in several cellular processes such as DNA repair, cellular senescence, and differentiation. Zeitlin and his colleagues showed that both laser- and nuclease-induced DNA double-strand breaks (DSB) are sufficient to recruit overexpressed CENP-A in a variety of human and mouse cancer-cell lines (37), which proposed a role for CENP-A in DSB repair. However, a recent study observed little or no endogenous CENP-A recruitment at sites of DNA damage induced by radiation in U2OS cancer cells (38). Similar results have also been reported in hPSCs (22). Although this divergence could be explained by the differences between the endogenous and exogenous expression of CENP-A, or the expression threshold of CENP-A reported in different studies, the contribution of CENP-A to DNA repair through its possible recruitment at sites of DNA damage remains to be explored further.

Cellular senescence is a phenomenon characterized by a stable cell-cycle arrest elicited in response to a variety of stressors including DNA damage. It requires functional p53 and pRB tumor-suppressor proteins and constitutes a potent anticancer mechanism (39). Interestingly, some studies in recent years suggested that chromosomal

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pathologies as a result of mitotic misregulation might also be involved in this process. Studies of fibroblasts derived from young, middle-aged, old-aged humans, and humans with Hutchinson-Gilford progeria revealed that the expression of CENP-A, along with other genes associated with regulating chromosomal processing and assembly, were decreased in cells from middle-aged, old-aged, and progeria versus young human participants (40). In human primary fibroblasts, knockdown of CENP-A by short hairpin RNA led to p53-dependent premature senescence (41). In addition to the CENP-A, its histone chaperone HJURP has also been reported to be downregulated in senescent cells (42). Therefore, it can be inferred from the above researches that CENP-A and/or HJURP reduction might induce p53-dependent senescence as a "self-defense mechanism" to keep centromere-defective cells from undergoing mitotic proliferation. Cell differentiation is another cellular process associated with cell-cycle withdrawal. A study found that CENP-A protein levels in chromatin increased with hPSC differentiation, suggesting that CENP-A might play a role in cell differentiation (22). As described above, the expression levels of CENP-A increase in the process of differentiation while decrease in the process of senescence.

Aberrant expression and ectopic localization of CENP-A in human cancers

As early as 2003, CENP-A has been documented to be upregulated in primary human colorectal-cancer tissues (43) and identified to correlate with breast-cancer stage (44). At present, the expression of CENP-A has been found to be overexpressed in as many as 20 types of solid cancers (**Fig. 2**; refs. 12, 16). Indeed, elevated CENP-A expression could now be used as a potential diagnostic and/or prognostic biomarker for many human cancers, such as epithelial ovarian cancer (45), lung adenocarcinoma (46, 47), estrogen receptor–positive breast cancer (48, 49), and osteosarcomas (13). Genetic analysis has been conducted in order to identify the mechanisms underlying CENP-A overexpression, however, the results indicate that the upregulated CENP-A expression is not due to the increased copy number of the gene (16). Consistent with this finding, another study indicated that CENP-A overexpression occurred at the transcriptional level in colorectal cancer (43). In the future, it would be interesting to test whether other types of regulation, such as posttranscriptional or posttranslational modifications, could contribute to CENP-A overexpression in human cancers.

Although CENP-A is demonstrated to be overexpressed in human cancers, little is known about its behavior and function in cancer cells. Under normal conditions, ectopic CENP-A assembled onto chromosome arms in early G1 is removed by replication fork progression (50), indicating another function of DNA replication to maintain epigenetically-defined centromere position and identity. However, in human cancers, the ectopic localization of CENP-A has also been observed along with its overexpression in many cases. Tomonaga and colleagues have demonstrated that upregulation of CENP-A in colorectal tissues results in its incorporation into the noncentromeric regions of chromatin (43). Similar noncentromeric localization of endogenous CENP-A has been observed in HeLa and HCT116 cells (51, 52). Although the association of CENP-A with the entire chromosomes could not be observed in colorectal tissues, overexpression of exogenous CENP-A in HeLa and HCT116 cells results in its incorporation into the entire length of chromosomes. The similarities and differences of CENP-A localization in tumor-cell lines and tumor tissues are somewhat confusing, but at least can be explained by the differences between the endogenous and exogenous expression of CENP-A.

Ectopic CENP-A—different choice for histone chaperone

While most studies have highlighted the complex and tight regulation that ensures proper centromere assembly during the cell cycle, noncentromere assembly in cancer models is still largely unknown. It has long been assumed that histone chaperones mainly function to carry histones, however, recent advances have shown that they may play an unanticipated role in disease progression including cancer (53). As the CENP-A chaperone, HJURP is responsible for CENP-A deposition at centromeres (54). Interestingly, the expression of HJURP is upregulated in several types of human cancers including breast cancer (55), lung cancer (56), ovary cancer (57), and glioblastoma (58). More importantly, *HJURP* mRNA levels are significantly correlated with *CENP-A* mRNA levels (55). This might be explained by the recent discovery that the tumor-suppressor p53 represses the expression of both CENP-A and HJURP through binding to elements in the promoters of these genes (17). The above observations raise a question as to whether HJURP overexpression in cancers results in ectopic deposition of CENP-A. Unlike CENP-A–overexpressing cells in which both centromeric and ectopic deposition of CENP-A were observed, cells with HJURP overexpression only showed an increased CENP-A centromeric localization (17). This result underscores the specificity of HJURP in mediating the centromere-specific deposition of CENP-A. Besides HJURP, the human Mis18 complex has also been identified as a critical factor for the centromeric localization of CENP-A. Mis18 seems to be the most upstream factor required for deposition of CENP-A since it precedes HJURP to centromeres and is required for HJURP recruitment to centromeres. As HJURP binds Mis18 and new CENP-A is deposited, the Mis18 complex is removed gradually from the centromere. In other words, the temporal regulation of Mis18 complex formation controls the time course of HJURP-mediated CENP-A deposition (59). Research results from a previous study showed that overexpression of Mis18 resulted in increased CENP-A deposition and the Mis18 coiled-coil mutants contributed to ectopic chromosome locus (60). There is ample reason to further explore the possible roles of Mis18 in ectopic localization of CENP-A in human cancers.

Notably, the correct localization of histone variants is controlled by the use of chaperones specific to each variant (61). Unlike HJURP, the DAXX histone chaperone, which is generally excluded from centromeric CENP-A nucleosomes, is H3.3-specific and responsible for the deposition of H3.3 into telomeres or pericentric heterochromatin (62). However, an earlier study has reported that DAXX associates with overexpressed CENP-A in HeLa cells, which contributes to the ectopic localization of CENP-A (63). Moreover, two other studies in colorectal cancer-cell line revealed the association between ectopic CENP-A and the H3.3 chaperones DAXX and HIRA (64, 65). In addition, they identified a distinct class of noncentromeric CENP-A nucleosomes: CENP-A partners with histone H3. The CENP-A/H3 nucleosomes form a stable octameric nucleosomal species. The above studies suggest that the abnormally-high CENP-A can assemble at noncentromeric regions of chromatin through a DAXX- or HIRA-dependent histone-deposition pathway instead of HJURP, indicating a shift in the usage of histone chaperones in human cancers (**Fig. 3**). Actually, it has been hypothesized that the balance between CENP-A and its "normal" chaperone is involved in regulating CENP-A localization (65). That's to say, relative to limited HJURP, excess levels of CENP-A tend to combine with alternative chaperones in the H3.3 pathway. Interestingly, a recent study showed that SUMOylation sites in the C terminus of Cse4 (the *budding yeast* CENP-A) regulates its interaction with histone chaperones, which facilitates the deposition of overexpressed

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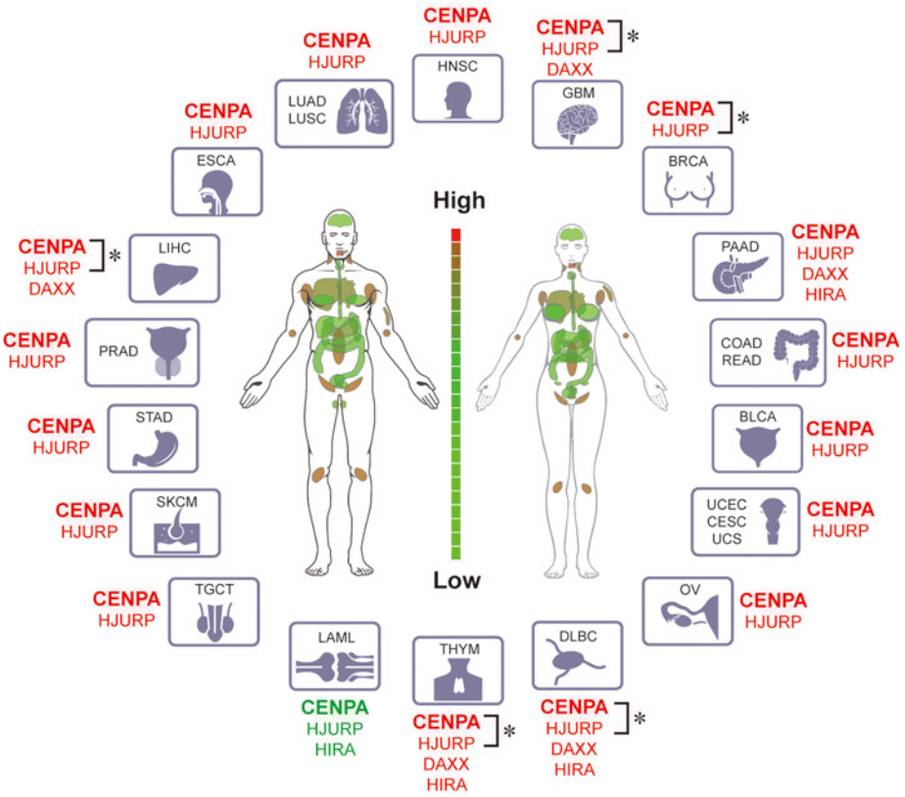

Figure 2.

Dysregulation of CENP-A and its chaperones in human cancers. The diagram in the center shows the mRNA expression levels of CENP-A in different tissues of males and females under normal conditions, which is downloaded from ProteomicsDB (https://www.ProteomicsDB.org). Red indicates high expression and green indicates low expression. The outer circle represents the dysregulation of CENP-A and its chaperones HJURP, DAXX, and HIRA in different types of human cancers. Red indicates that the respective gene is overexpressed in tumor versus normal tissue while green indicates that the respective gene is underexpressed. The asterisk represents that HJURP mRNA levels are significantly correlated with CENP-A mRNA levels in tumors. HNSC, head and neck squamous carcinoma; GBM, glioblastoma; BRCA, breast cancer; PAAD, pancreatic adenocarcinoma; COAD, colon adenocarcinoma; READ, rectal adenocarcinoma; BLCA, bladder cancer; UCEC, uterine corpus endometrial carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; UCS, uterine carcinosarcoma; OV, ovarian cancer; DLBC, diffuse large B-cell lymphoma; THYM, thymoma; LAML, acute myeloid leukemia; TGCT, testicular germ cell tumors; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; PRAD, prostate adenocarcinoma; LIHC, Liver hepatocellular carcinoma; ESCA, Esophageal carcinoma; LUAD, lung adenocarcinomas; LUSC, lung squamous cell carcinomas.

Cse4 into centromeric and noncentromeric regions (66). As a matter of fact, the histone chaperone DAXX also possesses two independent SUMO-interacting motifs (SIM) which play an important role in mediating protein–protein interactions (67). Therefore, it will be interesting to determine whether human CENP-A is SUMOylated and whether the SUMOylation of CENP-A regulates its interaction with DAXX and ectopic deposition.

In addition to binding to H3.3 chaperones, several studies have shown that CENP-A mislocalization recruits the kinetochore protein CENP-C to noncentromeric chromatin (52, 63, 64, 68). As is well known, CENP-C is a hub protein for kinetochore assembly and it normally localizes with CENP-A at the area of centromeric heterochromatin (69). However, in the case of CENP-A overexpression, this constitutive centromere protein is recruited to ectopic chromosomal regions with mislocalized CENP-A in different cell lines (52, 70, 71). Instead, overexpressed CENP-C does not assemble CENP-A to noncentromeric chromatin. Thus, it appears that the ability to assemble other components of the centromere-prekinetochore is an important property of CENP-A.

Based on the above findings, it would be critical to investigate the causal relationship between aberrant expression or localization of CENP-A and tumorigenesis. On the one hand, some tumor suppressors that are frequently mutated or disabled in human cancers, such as pRB and p53, are involved in CENP-A regulation. Studies supposed that CENP-A overexpression is a consequence of p53 or pRB inactivation (17, 72). On the other hand, the mechanism by which CENP-A overexpression and mislocalization contribute to tumorigenesis has been further explored. A recent study showed that CENP-A overexpression and mislocalization lead to chromosomal instability (CIN) in human cells with the latter as the major contributor (70). Furthermore, several studies have inferred that ectopic CENP-A not only tracks large regions found at chromosomal-rearrangement sites but also tracks small areas discovered at accessible chromatin and promoters (**Fig. 3**; refs. 12, 64). In colorectal cancer cells and tumors, a different type of CENP-A hotspots assembles at subtelomeric locations, including the region of 8q24/Myc (64), which has long been associated with tumorigenesis and chromosome instability. And

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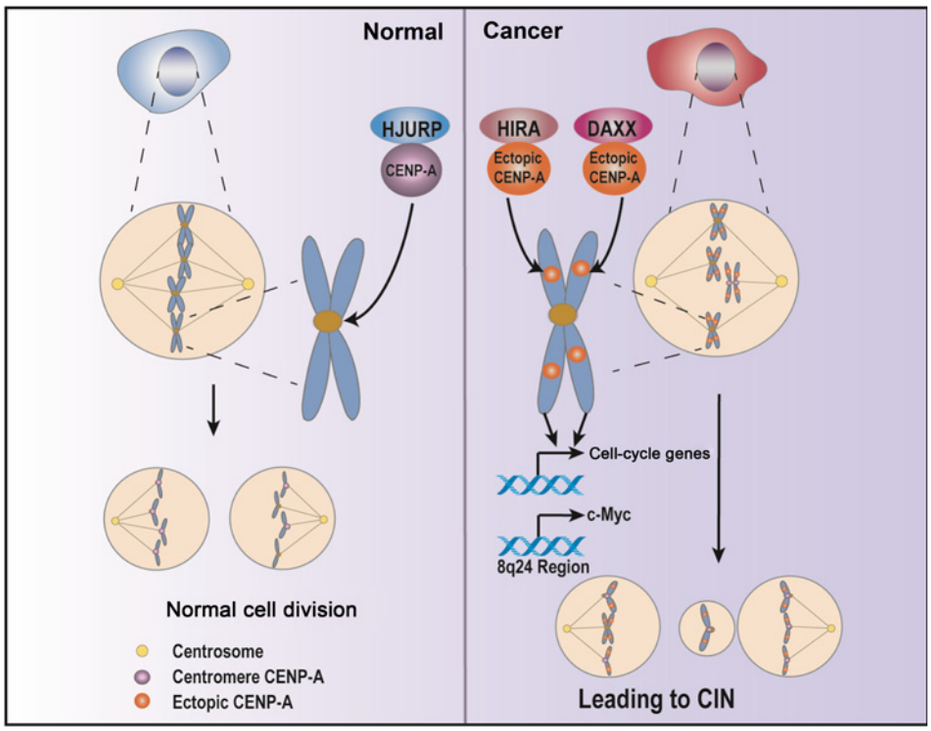

Figure 3.

CENP-A overexpression and mislocalization contribute to CIN and tumorigenesis. In normal cells, CENP-A is highly enriched at centromeres, which is required for accurate chromosome segregation during cell division. The histone chaperone HJURP mediates the centromere-specific deposition of CENP-A. However, in human cancers, abnormally high CENP-A can assemble at noncentromeric regions of chromatin through the H3.3 chaperone DAXX- or HIRA-dependent deposition pathway instead of HJURP. Moreover, ectopic CENP-A accumulates at subtelomeric chromosomal locations, including the 8q24/Myc region, or binds gene regulatory elements, such as TSS of cell-cycle–related genes. The overexpression and mislocalization of CENP-A altogether contribute to chromosomal instability.

CENP-A occupancy at the 8q24 locus correlates with amplification and overexpression of the Myc oncogene (65). It has also been identified that ectopic CENP-A binds transcriptional start sites (TSS) of genes important for cell-cycle progression, such as CDC25C (12). Taken altogether, the above studies provide direct evidence that overexpression and mislocalization of CENP-A could contribute to tumorigenesis.

The regulation of oncogenic viruses on CENP-A and its chaperone

Considering the relationship between CENP-A and human cancers, it will be meaningful to figure out whether oncogenic viruses can modulate the aberrant expression and localization of this centromere protein. Although current research on this issue is limited, there are still some important evidences to suggest that certain viruses, including Hepatitis B virus (HBV), human papillomavirus (HPV) and HSV at least, might take part in the regulation of this histone variant and its chaperone (**Fig. 4**). Previous study has shown that HBV X protein (HBx) mutant upregulates the expression of CENP-A mRNA and protein in hepatoma cells (73). Although the direct interaction between HBx and CENP-A did not exist, it is concluded that overexpression of CENP-A is closely associated with HBx COOH mutation. In addition, a nonsynonymous SNP located in the *HJURP* gene was found among Chinese populations that were infected with HBV (74). Interestingly, while this SNP correlates with lower expression of HJURP at the mRNA and protein level, it associates with the susceptibility to hepatocellular carcinoma. The dual roles played by HBV are not contradictory, but rather complementary since the insufficient levels of HJURP relative to CENP-A might promote CENP-A binding to chaperones in the H3.3 pathway and mislocalization.

Aneuploidy is one of the characteristic features of HPV-infected cells (75). A number of studies have shown that the high-risk HPV 18 and HPV58 E7 proteins bind to CENP-C, while low-risk HPV4, HPV6, and HPV11 E7s do not (76–78). Given that the interaction between E7 and CENP-C can inhibit CENP-A, CENP-B, CENP-C complex from binding to centromeric-satellite DNAs and thus cause aneuploidy, the different ability of high-risk and low-risk HPV E7s to bind to CENP-C reflects the different oncogenic potential of HPV types. These studies provide direct evidence that the ability of oncogenic viruses to modulate the aberrant expression and localization of centromere proteins correlates with their oncogenic capability.

Accumulating evidence suggests that HSV-1 is a possible etiological factor in causation of cancer and precancer (79, 80). It is worth noting that HSV-1 could also destabilize the centromeric chromatin structure due to the viral protein ICP0. In addition to CENP-A as mentioned above, CENP-B (81) as well as several major components of the CCAN including CENP-H, CENP-I, CENP-N, CENP-C (82) were all identified to be targeted for proteasomal degradation by ICP0. Like all herpes viruses, HSV1 is able to produce lytic or latent infections. This RING finger domain-containing protein has been reported to play a critical role in controlling the balance between the lytic and latent outcomes of HSV-1 infection (36). Although ICP0 is essential for reactivation, it is still obscure whether and how ICP0-induced centromere destabilization perform a function in the reactivation of HSV-1 from latency. Taken together, the ability of different viruses to regulate centromere protein and its chaperone is achieved by several different mechanisms. More researches about the regulation of viruses on centromere protein and its chaperone will be needed in the future.

The emerging role of CENP-A as a target for cancer therapy

As discussed above, growing evidence has identified that CENP-A overexpression and mislocalization is one of the driving forces of cancer development. Therefore, targeting dysregulated CENP-A might represent an attractive strategy for cancer therapy. More recently, an interesting hypothesis has been proposed. That is, cells

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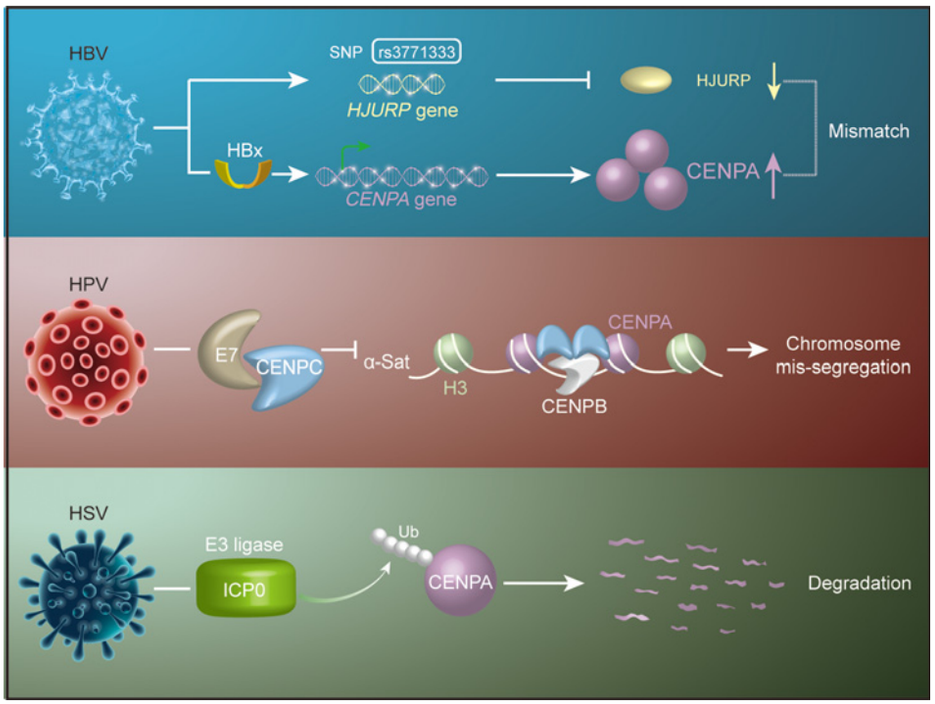

Figure 4.
The regulation of oncogenic viruses on CENP-A and its chaperone. Human oncogenic viruses HBV, HPV, and HSV take part in the regulation of CENPA and its chaperone. In HBV-infected hepatoma cells, HBx mutant upregulates the expression of CENP-A. Furthermore, an SNP that correlated with lower expression of HJURP is found in HBV-infected populations. The insufficient levels of HJURP relative to CENP-A might promote CENP-A binding to chaperones in the H3.3 pathway. The high-risk HPV18 and HPV58 E7 proteins can bind to CENP-C, which inhibit CENP-A, CENP-B, CENP-C complex from binding to centromeric-satellite DNAs and cause chromosome missegregation. During HSV-1 infection, the RING-finger E3 ubiquitin ligase ICP0 induces the proteasome-dependent degradation of CENP-A protein, leading to the loss of CENP-A from centromeres and cell-cycle arrest.

undergoing malignant transformation need increasing CENP-A deposition and centromere propagation to sustain high proliferation rates, while nonmalignant cells can proliferate with low levels of CENP-A, which can be concluded as a kind of "epigenetic addiction" of CENP-A in cancer (17). According to this hypothesis, inhibiting CENP-A deposition will impede progression in an existing tumor while inducing milder consequences in surrounding healthy tissue that has a lower rate of proliferation. Indeed, knockdown of CENP-A–induced G_0–G_1 cell-cycle arrest and apoptosis, and also inhibited the *in vitro* migration and invasion of lung adenocarcinoma A549 and PC-9 cells (83). In contrast, CENP-A deletion only caused senescence-like proliferation arrest with the majority of cells appeared to be in the G2 phase in primary human diploid-lung fibroblasts TIG3 cells (41). Similarly, in prostate cancer cells, CENP-A loss led to a profound growth-inhibitory effect on 22Rv1, LnCaP, and DU145 with cell-cycle arrest at the G1 phase, while 957E-hTERT benign prostate-epithelial cells depleted of CENP-A did not demonstrate significant proliferative changes (12). These studies showed a promising therapeutic potential of targeting CENP-A for cancer therapy.

Taken all together, there seem to be at least three new therapeutic strategies. The first one is to suppress the levels of CENP-A itself. In terms of molecular structure, CENP-A possesses a COOH-terminal histone-fold domain that is similar to histone H3 and a highly variable NH2-terminal domain. The latter could be used as a target to design anticancer drugs. Although an antibody against the NH2-terminal peptide of human CENP-A (residues 3–17) has been generated (84, 85), the inhibitor of CENP-A has not been reported yet. At present, most of the studies suppressed CENP-A production through siRNA-mediated depletion, so it would be particularly important to discover pharmaceutical compounds to inhibit this centromere protein in the future.

Secondly, with the in-depth study about the mechanisms of CENP-A mislocalization, it seems likely that blocking cancer-specific interactions between histone variants and chaperones, such as DAXX binding to CENP-A, or inhibiting the recruitment of CENP-C to noncentromeric chromatin by CENP-A, can also serve as a potential strategy to particularly attack cancer-specific networks while sparing normal cells. For example, if the SUMOylation of CENP-A indeed regulates its interaction with DAXX and ectopic deposition, it will be interesting to explore the inhibitor of CENP-A SUMOylation.

At last, considering the effect of CENP-A on DNA repair, it is reasonable to speculate that CENP-A misregulation could be a significant element determining increased resistance to tumor treatment or recurrence since ectopic CENP-A provides an important survival advantage in the presence of ionizing radiation and DNA-damaging agents camptothecin (CPT; ref. 63). Therefore, additional CENP-A targeted therapy might be considered in patients with tumors with radiation resistance or DNA-damaging chemotherapeutic agent resistance.

Conclusions and Perspectives

Within the past few years, extensive efforts have been devoted to study the expression of CENP-A in human cancers. It is now clear that both CENP-A and its chaperone HJURP are upregulated in various types of cancers, and their dysregulation could be associated with prognosis in patients with cancer. Evidence is emerging that abnormally high CENP-A can assemble at noncentromeric regions of chromatin through the H3.3 chaperone DAXX or HIRA instead of the HJURP-dependent histone deposition pathway in cancers. Notably, in certain types of cancers related to virus infection, oncogenic viruses can modulate the aberrant expression and localization of CENP-A, shedding light on the molecular mechanisms of tumorigenesis that are employed by these viruses.

As discussed above, this review systematically summarized the current knowledge on the relationship between aberrant expression and ectopic localization of CENP-A and tumorigenesis, as well as the mechanism of the ectopic deposition of CENP-A in cancers. Detailed insights into the molecular mechanisms of centromere dysregulation

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and the function of centromere proteins will help to improve our understanding of cancer and to find a new way to fight it. Finally, we will suggest the future direction of studies on the CENP-A topic. First of all, although CENP-A overexpression at the transcriptional level has been described in several human cancers, studies investigating whether posttranscriptional regulation, such as microRNAs, or posttranslational regulation, such as SUMOylation, could contribute to CENP-A overexpression or ectopic deposition in human cancers are needed in the future. Next, studying how and when CENP-A associates with DAXX and HIRA will greatly improve our understanding of the underlying mechanisms of CENP-A mislocalization and thus represents one of the future directions. In addition, since most studies about CENPA thus far have focused on its expression and mislocalization in tumorigenesis, it is also important to understand the structural change of the chromatin during cancer progression.

Authors' Disclosures

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