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Cdt1 and Geminin in cancer: markers or triggers of malignant transformation?

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1. ABSTRACT

Cdt1 and its inhibitor Geminin are important regulators of replication licensing. In normal cells, a critical balance between these two proteins ensures that firing of each origin along the genome will take place only once per cell cycle. Cdt1 overexpression in cell lines and animals leads to aberrant replication, activates DNA damage checkpoints and predisposes for malignant transformation. Geminin inactivation mimics the effects of Cdt1 overexpression in cells and generates mitotic defects and abnormal chromosome segregation. Aberrant expression of Cdt1 and Geminin is thus linked to DNA replication defects, aneuploidy and genomic instability. These traits are considered integral to precancerous states and essential elements for malignant transformation. Moreover, Cdt1 and Geminin expression is deregulated in human tumor specimens and Cdt1 and Geminin may represent novel markers useful for cancer diagnosis and prognosis.

2. INTRODUCTION

The initiation of DNA replication must be tightly controlled to ensure that the genetic material will be duplicated fully and only once per cell cycle. From yeast to humans, initiation of DNA replication requires the prior assembly onto chromatin of a multiprotein machinery, called the pre-replicative complex (pre-RC), which is found on chromatin from the end of mitosis until S-phase. Successful assembly of the pre-RC licenses chromatin for a new round of DNA replication (for reviews see (1-4)). Evidence from both lower and higher eukaryotes has shown that licensing takes place at the end of M phase by the sequential loading onto the origins of replication of the Origin Recognition Complex (ORC), Cdc6 and Cdt1, which in turn recruit the six-subunit minichromosome maintenance complex (MCM2-7), believed to act as the replicative helicase (Figure 1). At the onset of S phase, as S-phase cyclin dependent kinase (S-Cdk) activity increases,

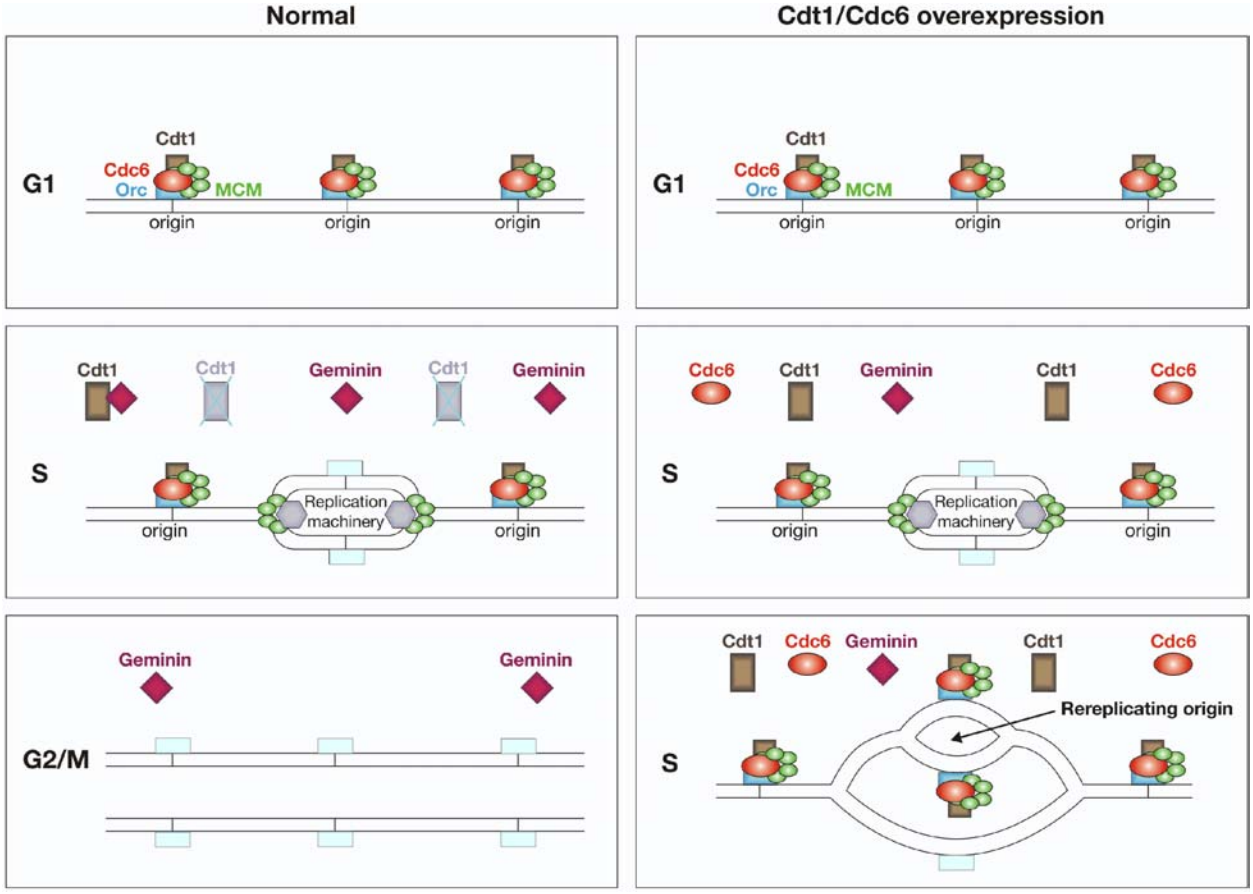

Figure 1. In normal cells (left panels), a multiprotein complex (pre-replicative complex) consisting of the origin recognition complex (ORC), Cdc6, Cdt1 and MCM proteins is formed on mammalian origins of replication during the G1 phase. Origin firing inactivates the pre-replicative complex, while MCM proteins are moving along with the replication machinery. During S phase and in G2, pre-replicative complex subunits are subject to negative regulation: Cdt1 is proteolytically degraded and remaining amounts are inhibited by Geminin; Cdc6 is subject to proteolysis and nuclear export, while ORC complex subunits are negatively regulated through post-translational modification, changes in affinity for origin binding or proteolysis. Pre-replicative complex subunits become functional again in late M phase. In abnormal situations, where Cdt1 and/or Cdc6 are overexpressed (right panels), untimely activation of the pre-replicative complex leads to refiring of origins in the same cell cycle (re-replication).

DNA synthesis is initiated at specific origins. In normal cycling cells, pre-RCs disassemble and licensing factors are inactivated concomitantly with replication initiation, in a temporally and spatially controlled fashion, so as to ensure that re-initiation of replication is prevented during the same S phase and the G2 phase of the cell cycle. The pre-RC is ready to assemble anew only after the end of mitosis.

Cdt1 was identified as an essential factor for the loading of MCM proteins onto chromatin in both lower and higher eukaryotes (5-10). The Cdt1 protein is tightly regulated during the cell cycle and its regulation is critical for once per cell cycle replication. It accumulates during the G1 phase of the cell cycle (11), while upon replication initiation, Cdt1 is targeted for ubiquitin-dependent proteolysis (12-16). In human cells, Cdt1 is targeted for degradation by two E3 ubiquitin ligases, SCF-Skp2 and DDB1-Cul4-Cdt2, the first requiring cyclin-dependent kinase mediated phosphorylation of Cdt1 and the second Cdt1 binding to PCNA (17-19).

Higher eukaryotes have developed an additional mechanism to regulate Cdt1 inactivation. Geminin binds tightly to Cdt1 and can prevent MCM protein loading onto chromatin (20, 21). The Geminin protein accumulates in S and G2 and is degraded by the anaphase promoting complex during mitosis (22). Cdt1 is believed to be inactive when bound to an excess of Geminin, and is only allowed to form part of the pre-RC at the end of mitosis when Geminin levels decrease due to degradation and any remaining Geminin is inactivated (23, 24). The cellular balance between Cdt1 and Geminin is thus critical since the amount of Cdt1 that is not degraded should not exceed the amount of Geminin needed to inhibit its activity. This balance ensures that an illegitimate pre-RC will not be formed following initiation of DNA replication and until mitosis is completed thus preventing re-replication of

already fired origins. Xouri *et al.* showed that in human cell lines Cdt1 interacts dynamically with chromatin throughout the G1 phase, suggesting that re-iterative licensing may take place throughout G1 (25), while Lutzmann *et al.* showed that a Geminin/Cdt1 complex could either promote or inhibit licensing, depending on its stoichiometry (26), underscoring the need of a tight spatio-temporal control of Cdt1 and Geminin levels and activities in order to permit once per cell cycle replication.

Intriguingly, Geminin appears to have a role in addition to regulating the cell cycle through interactions with Cdt1. Geminin has been shown to bind directly to transcription factors and chromatin remodeling activities implicated in cell fate decisions, such as Six3 (27), homeobox transcription factors (28), polycomb family members (28) and the SWI/SNF chromatin remodeling components Brahma and Brg1 (29). Through these interactions, Geminin is believed to regulate the transcriptional programs which lead to cell specification and differentiation during development. Existing evidence functionally links Geminin to the development of the nervous system in Xenopus (29), fish (27) and chicken (28). Therefore, through balanced interactions with Cdt1 on one hand, and transcriptional regulators on the other, Geminin appears to lie at the heart of a cell's decision to proliferate versus differentiate. Interestingly, a link between Geminin and apoptosis was also recently identified: Geminin is specifically cleaved by caspase 3 during apoptosis, generating fragments which bind differentially to Geminin's partners and could differentially promote apoptosis or differentiation (30).

Licensing of replication origins is an evolutionary conserved step in the control of cellular division and genomic integrity. Increasing lines of evidence suggest that Cdt1 and its inhibitor Geminin are important regulators of pre-replicative complex formation (see accompanying review (31)). In addition, Geminin has been implicated in developmental decisions (see accompanying review (32)). In the present manuscript we will discuss data suggesting a link between Cdt1 and Geminin and the molecular pathway of carcinogenesis. In addition we will discuss the use of these two molecules as biomarkers for tumor diagnosis.

3. A ROLE FOR CDT1 AND GEMININ IN REGULATING GENOMIC INTEGRITY AND CANCER PROMOTION IN TUMOR CELLS

Genomic instability is a whole-mark of cancer cells. Around 50% of all tumors exhibit aneuploidy, evident as accumulation of additional copies of genes, genomic regions or whole chromosomes as well as gross chromosomal rearrangements. In addition, tumor cells are often genetically unstable, changes in their genetic content accumulating in faster rates than in normal cells. This genetic/genomic instability is believed to lie at the heart of the acquisition of new traits by cancer cells. Genomic instability could arise due to the loss of control mechanisms which operate during the normal cell cycle. In eukaryotic cells, thousands of replication origins must fire at the correct point in time in order to allow rapid and accurate duplication of the genetic material. Origin re-firing within the same cell cycle would generate over-replicated DNA segments that could cause genomic instability (Figure 1). Towards the direction of enlightening the molecular events contributing to aberrant replication that could generate aneuploidy, unicellular eukaryotes have been employed as a model system to identify proteins involved in once per cell cycle replication through genetic screens. Studies in higher eukaryotes have subsequently shown that the pathways are highly conserved in evolution.

In fission yeast, Cdc18 (the Cdc6 homologue in this species) was the first pre-RC component shown to induce massive genome over-replication due to origin re-firing when ectopically expressed (33). It was later shown that Cdt1 would enhance Cdc18 mediated re-replication (5), even in G2 arrested cells (34).

In metazoa, Cdt1 ectopic expression alone was shown to be sufficient to cause re-replication in C. elegans (35), Drosophila (36), Xenopus (15, 37) and humans (38) (Figure 1). Vaziri *et al.* showed that Cdt1 overexpression in a lung cancer cell line deficient for p53 induces re-replication and this is enhanced by concomitant Cdc6 overexpression (38). Analysis of the localization of re-replication events along the chromosomes showed that they are scattered throughout the genome and correlate with regions replicating early during a normal S-phase. In a p53 wild type lung cancer cell line, Cdt1 and Cdc6 ectopic expression activated the DNA damage checkpoint through phosphorylation of chk2, phosphorylation and stabilization of p53 and subsequent cell cycle arrest, possibly due to p21 accumulation, while evident re-replication was prevented (38). Subsequent studies showed that Cdt1 ectopic expression in a number of tumor and primary cell lines induces DNA damage likely to be double strand brakes as is manifested by phosphorylation of the histone variant H2AX, and leads to induction of the ATM-Chk2 pathway in cycling and quiescent cells, even in the absence of evident genome over-replication ((39) and Z. Lygerou and S. Taraviras unpublished data). Mechanistically the generation of local or extended re-replication can generate collisions between replication forks that generate DNA breaks. Indeed, Davidson *et al.* showed, using Xenopus egg extracts, that addition of Cdt1 leads to re-replication of G2 chromatin and appearance of double stranded DNA fragments, consistent with head-to-tail fork collisions (40). This appears to be the main mechanism of DNA damage due to Cdt1 overexpression, though alternative possibilities cannot be excluded, such as defects in sister chromatin cohesion due to the presence of more than two daughter strands (40). Interestingly, an additional link between Cdt1 and the DNA damage response exists: Cdt1 is rapidly targeted upon detection of DNA damage through proteolysis from the DDB1/cul4/cdt2/PCNA complex. This mechanism, which is ATM/ATR independent, has been suggested to safeguard genomic integrity and prevent re-replication while DNA repair is in progress (41, 42).

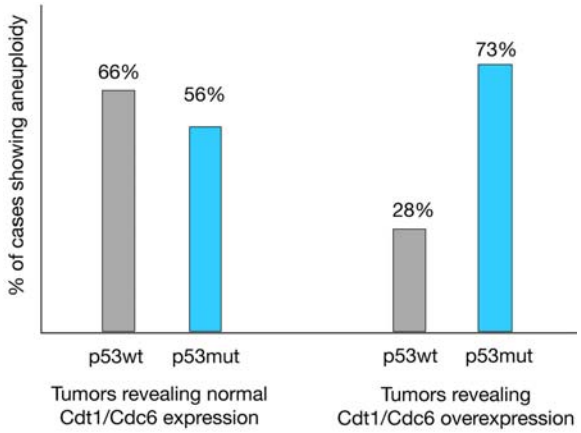

Figure 2. Cdt1/Cdc6 overexpression is correlated with aneuploidy in Non-Small-Cell-Lung carcinomas bearing mutations in p53. In Non-Small-Cell-Lung carcinomas exhibiting Cdt1 and/or Cdc6 overexpression, 73% of tumors carrying a mutant p53 (p53mt) exhibit aneuploidy, as compared to 28% carrying wild type p53 gene. However, NSCLC tumors with normal Cdt1/Cdc6 expression, % aneuploidy is independent of p53 status (66% in the subset of tumors with p53wt and 56% in those with mutant p53) (for more details see (45)).

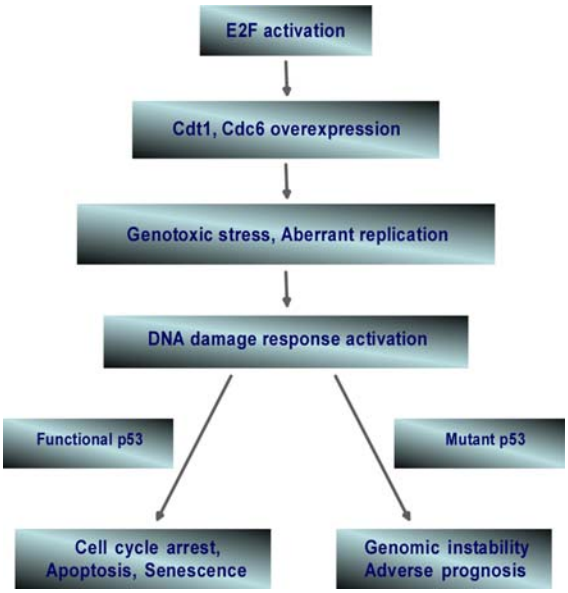

Figure 3. Working model for Cdt1 contribution to cancer development. Hyperactivation of E2F transcription factors can contribute to Cdt1 and Cdc6 overexpression. Ectopic expression of Cdt1 and Cdc6 would lead to refiring of origins already replicated. Re-replicated DNA is sensed by the cell as genomic stress and activates the DNA damage response. In the presence of a functional p53 protein, cellular safeguard mechanisms against cancer (cell cycle arrest, apoptosis, senescence) will be triggered. In the absence of functional p53, survival of cells carrying genomic alterations due to partial or extended re-replication will permit selection of cells with a proliferative advantage. Cdt1 and Cdc6 misregulation during malignant transformation could thus contribute to cancer development and act as a selective pressure for loss of p53.

Could over-replication caused by Cdt1 overexpression predispose for malignant transformation and be linked to the genomic instability observed in tumor cells? The answer appears to be yes. Cdt1 aberrant expression can predispose cells for malignant transformation, suggesting a putative oncogenic potential for Cdt1. Adentson *et al.* identified Cdt1 as a putative oncogene activated by retroviral DNA integration in an immortalised primitive erythroid cell line (43). Moreover they have shown that NIH3T3 cells overexpressing Cdt1 are forming tumors in Rag2-/- mice. Tatsumi *et al.* showed that ectopic expression of Cdt1 in normal human fibroblasts generates chromosomal instability (39). Seo *et al.* provided further *in vivo* evidence of the ability of Cdt1 to contribute to tumorigenesis. Overexpression of Cdt1 in T cells using Lck promoter elements led to the development of lymphoblastic lymphomas in the absence of p53 (44). So Cdt1 ectopic expression has the ability to lead to genomic instability and predispose for malignant transformation. Is this a pathway observed in human tumors? Karakaidos *et al.* offered support that this may indeed be the case, at least in non-small cell lung carcinomas (45).

Karakaidos *et al.*(45) analyzed a panel of non-small-cell-lung carcinomas (NSCLCs) and adjacent healthy tissue pairs for Cdt1 and Cdc6 expression. Cdt1 was overexpressed at both the mRNA and protein level as compared to normal adjacent tissues in nearly 50% of NSCLCs examined. Cdt1 overexpression was strongly correlated with Cdc6 overexpression in an overall 33% of patients. Furthermore, it was further established that the lung carcinomas that overexpressed Cdt1 also demonstrated increased E2F1 activity as judged by increased levels of E2F1 expression, loss of heterozygocity for Rb and/or hyperphosphorylation of the Rb protein. Confirmation of the functional significance of this correlation was presented by the identification of functional E2F binding sites at the Cdt1 promoter region. Most intriguing were the findings that overexpression of Cdt1/Cdc6 in combination with p53 mutation showed a strong correlation with aneuploidy, higher proliferation rate and lower percentage of apoptosis (Figure 2). Karakaidos *et al.* (45) therefore presented *in vivo* data of a possible signaling network participating in lung cancer development. Activation of E2F/Rb pathway may direct, among other factors, Cdt1/Cdc6 overexpression and depending on the p53 status, contribute to tumor development/promotion or apoptosis (Figure 3).

In higher eukaryotes accurate regulation of Cdt1 expression is of major importance for preventing re-replication and for the maintenance of genomic stability. Does inactivation of Cdt1's inhibitor, Geminin, mimic the effect of Cdt1 overexpression in cells? Several studies have shown that loss of Geminin generates re-replication (46-48). Genome over-replication is detected in several different human cell lines following Geminin inactivation by RNA interference (47, 48), though not in all studies (49). In contrast to Cdt1 overexpression however, this over-replication has been reported to be independent of p53 status (48). Moreover, depletion of Geminin in HCT116 cells triggers a G2/M checkpoint through an initial

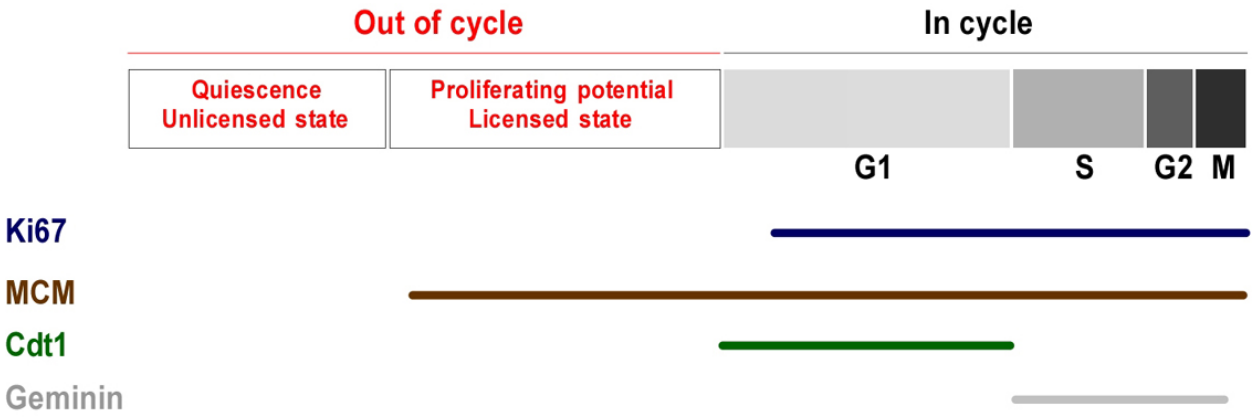

Figure 4. Proteins participating in pre-replicative complex formation are expressed in distinct phases of the cell cycle, while there are absent from quiescent unlicensed cells. Analysis of MCM, Geminin and Cdt1 expression in conjunction with established pathology markers like Ki67 can extend our ability to characterise the actively proliferating, licensed but not actively proliferating and not proliferating quiescent pool of cells in each tumor.

activation of ATR/Chk1 pathway and a subsequent activation of ATM/Chk2 (50). Furthermore, genetic inactivation of the mouse Geminin gene leads to very early death during embryogenesis and cells exhibit characteristics of over-reduplication and DNA damage, possibly via Cdt1 hyperactivity (51, 52). In addition to its role in regulating once per cell cycle replication, Geminin has been proposed to be involved in maintaining centrosome integrity. Tachibana *et al.* reported that depletion of Geminin by siRNA in human tumorigenic cell lines causes centrosome over-duplication, mitotic defects and abnormal chromosome segregation, and suggested that down-regulation of Geminin expression might contribute to aneuploidy due to mitotic defects (53).

Geminin overexpression, on the other hand, has been shown to arrest cell proliferation. When a stable form of Geminin was overexpressed in osteosarcoma, breast and colon cancer cell lines it was able to induce an arrest of cell cycle progression and proliferation (54-56). Overexpression of this stable form of Geminin decreased MCM protein loading onto chromatin (54, 56) and arrested cells at the G1 (55, 56) or early S phase (54) of the cell cycle. The anti-proliferating effect of stable Geminin was also verified *in vivo*. Nude mice bearing xenografts of mutant Geminin overexpressing cells showed a dramatic reduction in tumor induction and growth as compared to wild type Geminin cells (56). Interestingly, normal human fibroblasts appear to respond differentially to Geminin ectopic expression in comparison to tumor-derived cells (54). While normal cells arrested before S-phase, tumor-derived cell lines progressed into S-phase accompanied by an increase in cell death (54). This latter observation has led to the suggestion that molecules mimicking Geminin's function could constitute promising tumor-specific drugs.

Taken together, a number of lines of evidence suggest that a tight balance between Cdt1 and Geminin is crucial for maintaining genome stability, and that defects in this balance could predispose cells for malignant transformation.

4. GEMININ AND CDT1 AS MARKERS FOR CANCER PROGNOSIS

The increasing need for specific and accurate diagnostic markers in human cancer has led to recent evaluation of proteins that participate in pre-replicative complex formation as putative markers of the proliferative potential of cells. Accumulating evidence suggests that licensing factors may constitute highly promising markers in the diagnostic evaluation of different cancers. Emerging evidence in the last few years implicates MCM proteins as novel biomarkers in cancer diagnosis (reviewed in (57)). MCM proteins are expressed throughout the cell cycle and in cells which have replicative potential even if not actively proliferating (Figure 4). It has been proposed therefore that use of MCM proteins might represent a better marker for the distinction of cycling cells and cells with a proliferative potential, from cells in "out of cycle" states. Cdt1 and Geminin which are expressed at different cell cycle phases of actively proliferating cells (11) and are down-regulated upon cell cycle exit (58), could similarly function as good indicators of the proliferative state of cells in a tumor.

Given the inhibitory role of Geminin during the cell cycle, it was initially believed that in cancer cells, which are characterized by unrestricted proliferative potential, Geminin would be down-regulated. However, Geminin mRNA and protein was overexpressed in several tumorigenic cell lines as compared to normal cells (55, 58) and Geminin was highly expressed in invasive breast, cervix and colon carcinomas compared to normal tissues where it was barely detectable (55). In normal tissues, Geminin was expressed in proliferating cells of the mouse intestinal epithelium (58) and the developing central nervous system (59) similarly to Cdt1. Moreover, Geminin was found to be down-regulated, together with Cdt1, when cells exit the cell cycle and enter the quiescent state (58, 60) while significant down-regulation of Geminin was observed during *in vitro* differentiation of intestinal epithelial Caco-2 cells (61). Therefore, rather than functioning as a tumor suppressor, Geminin appears a good

indicator of the proliferative state and could therefore be used as a proliferation marker.

During the last few years, Geminin levels have been extensively characterized and compared to conventional proliferation markers in a number of neoplasms of different origin. In most of these studies, Geminin expression was shown to correlate with disease stage, and the ratio of Geminin to Ki67 expression has been suggested as a valuable indicator of the fraction of cells actively cycling (62).

Geminin and Ki67 expression was examined by immunohistochemistry in 24 tumors of large B cell lymphomas. Plotting Geminin and Ki-67 labeling index in malignant cells revealed a direct correlation, suggesting that Geminin expression correlates with increased proliferation (55). In a different study, low-growth fraction lymphomas and high-growth fraction lymphomas were examined for Geminin and Ki67 protein expression (63). In low-fraction lymphomas, Ki67 and Geminin expression was low, while in high growth lymphomas both markers were expressed at high and medium/high levels respectively. The Ki67 to Geminin ratio was high in aggressive neoplasms suggesting a possible diagnostic use in differential diagnosis (63).

In breast cancer, Geminin expression levels were used in conjunction with Ki67 and MCM-2 in order to more accurately characterize breast cancer cases. Immunohistochemical analysis revealed that Geminin is overexpressed in malignant breast tissue when compared to normal tissue where it is nearly undetectable. In a large number of invasive breast carcinomas analyzed, Geminin expression was increased and correlated with adverse clinical outcome and increased tumor grade (62, 64).

An increase in the levels of Geminin expression was observed in colon cancer as tumors advanced from adenomas to invasive and metastatic lesions. Geminin expression was significantly correlated not only with tumor progression but also with disease stage and depth of invasion (61). Geminin was also analyzed in CNS tumors oligodendrogliomas and astrocytomas (65-67). Immunohistochemical analysis in 55 oligodendrogliomas reveals an increased percentage of cells expressing Geminin that correlates with higher grade tumors (grade III vs grade II). Furthermore, Geminin expression showed a strong quantitative relationship with MCM2 labeling index while the ratio Geminin/Ki67 was significantly higher in grade III than in grade II tumors. No differences were observed on Geminin expression levels in the two molecular types of oligodendrogliomas defined by 1p and 19q deletions (65, 67). In a retrospective study of 51 patients with high-grade astrocytic tumors Geminin expression increased significantly as tumors progressed from grade I to grade III, IV (66). In renal cell carcinoma Geminin was expressed in a limited number of cells and although an association with increased disease-free survival time was detected, MCM2 and Ki67 expression were more representative of disease progression (68). A study from the Melanoma Group of the European Organization for Research and Treatment of Cancer that combined microarray and immunohistochemical analysis identified 254 genes associated with distant metastasis-free survival in melanoma patients. Geminin and MCM proteins were amongst the proteins identified for which increased expression correlated with reduced survival (69). Gene expression microarray analysis in search of genes that are up-regulated in biliary tract carcinomas identified Geminin among the genes that show a three-fold higher expression levels compared to normal epithelial tissue. Subsequent immunohistochemical analysis in biliary tract adenocarcinoma demonstrated diffuse or focal nuclear overexpression of Geminin protein, while normal biliary epithelium was negative (70).

Cdt1, has only recently started being evaluated as a prognostic marker in human cancers, mostly due to the lack of suitable antibodies for extensive analyses. Karakaidos *et al.* (45) determined Cdt1 mRNA levels in NSCLCs and showed that Cdt1/Cdc6 overexpression in the presence of mutant p53 correlates with adverse survival prognosis, while p53 status did not make any significant difference in survival prognosis in patients where no increase in Cdt1/Cdc6 expression was observed. Interestingly, a statistically significant correlation was found between Geminin and Cdt1 overexpression in NSCLC. This is reminiscent of studies in cells in culture, where induction of Geminin expression was observed after Cdt1 overexpression (38), suggesting the existence of a positive feedback loop between Cdt1 and its inhibitor Geminin. In a different study, 33 cases of mantle cell lymphoma (MCL) and 10 cases of reactive tonsils were examined for the mRNA expression of Geminin, Cdc6 and Cdt1. Cdt1 mRNA expression was significantly increased in MCL when compared to non-neoplastic tissues. A small subset of these tumors, which was characterized by a higher expression of Cdt1, Cdc6 and inactivation of p53/p14ARF pathway, showed a significantly higher number of chromosomal abnormalities (71). Enhanced Cdt1 protein expression was detected by immunohistochemistry in cancerous lesions as compared to non-neoplastic colonic epithelium (61). Furthermore Cdt1 expression was detected in the proliferating cells of the tumors, as assessed by PCNA co-localization. Nuclear Cdt1 was never co-localized with Cyclin A, while Geminin showed a complete overlapping pattern, suggesting that in this subset of tumors Cdt1 and Geminin maintained the phase specific expression described in tumorigenic cell lines (11, 58, 61).

Taken together, analyses in a number of human tumor specimens suggest that Geminin and Cdt1 constitute highly promising markers for prognosis. Geminin is highly expressed in different tumors and its increased expression correlates with the clinicopathological parameters of the tumor. Cell cycle phase specific expression of Geminin and Cdt1 is maintained in tumors and therefore their expression can reflect accurately the active fraction of proliferating tumor cells (Figure 4). Furthermore the combination of Geminin and Cdt1 expression with Ki67 expression, an established marker of actively proliferating cells, and MCM protein expression which reveals tumor cells that are

able to divide, could facilitate the discrimination between aggressively proliferating cells from the ones that have proliferative potential but are present in a prolonged licensed state, and from out of cycle cells (Figure 4). Further analysis is required, employing large numbers of tumor specimens, in order to establish the prognostic value of this set of markers for tumor progression.

5. PERSPECTIVES: GEMININ AND CDT1 AS PLAYERS IN THE MOLECULAR MECHANISMS OF CARCINOGENESIS

The maintenance of genomic integrity is an ultimate goal for the eukaryotic cell. DNA replication and chromosomal segregation are two physiological processes tightly controlled from lower eukaryotes to humans, ensuring accurate duplication and segregation of the genetic material. Genomic integrity in cancer cells is disrupted and tumor cells exhibit disequilibrium in the dosage of genes, with the ones that promote cell growth being amplified and the ones that suppress it being underrepresented. In addition, cancer cells are characterized by genomic instability which is believed to permit the acquisition of novel traits by cancer cells. In order to prevent carcinogenesis, cellular mechanisms are employed upon DNA and cellular stress in order to halt cell cycle progression, repair damage or direct cells to undergo programmed cell death or senescence. Recent studies provide evidence that activation of oncogenes in a precancerous lesion is accompanied by hyperproliferation and DNA hyper-replication (72, 73). Replication stress is considered as an integral part of precancerous states and is believed to trigger the DNA damage response and contribute to genomic instability (74, 75).

Studies on tumorigenic cell lines and human specimens suggest that Cdt1 aberrant expression could be implicated in generating replication stress and inducing DNA damage and chromosomal instability. Therefore, Cdt1 might constitute a potential target of oncogene activation on early precancerous lesions and therefore be involved in early steps of carcinogenesis and the generation of genomic instability, thereby accelerating cancer development. Several independent lines of evidence suggest that proteins participating on the formation of the pre-replicative complex in addition to Cdt1 are implicated in the molecular mechanisms of carcinogenesis. Mutations in MCM4 cause chromosomal instability and mammary adenocarcinomas in mice (76) while aberrant expression of Cdc6 is able to promote oncogenesis through repression of the INK4/ARF locus (77).

Although the role of Cdt1's partner, Geminin, in tumor development remains puzzling, it is now clear that it does not behave as a classical tumor suppressor gene. Contrary to its inhibitory role on the formation of the pre-replicative complex, Geminin is overexpressed in human cancers and its expression often defines more accurately the proliferative fraction of the tumor cells. In different experimental systems, absence of Geminin has been shown to promote genome over-replication through a Cdt1 related mechanism. Intriguingly, increasing lines of evidence introduce a positive role for Geminin on proliferation control. Given the multiple binding partners of Geminin during the cell cycle and during development, further analysis will be required to shed light into a possible Geminin involvement in tumor development and progression.

6. ACKNOWLEDGEMENTS

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Note added in proof: While this manuscript was in proof a manuscript by Liontos et al. Cancer Res. 2007 Nov 15;67(22):10899-909 was published, providing evidence that Cdt1 and Cdc6 overexpression contributes to the mechanisms leading to malignant transformation.

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