Breast Cancer RESEARCH

EDITORIAL

Spotlight on Geminin

Melvin L DePamphilis*

See related research by Gardner *et al.*, http://breast-cancer-research.com/content/13/2/R53

Abstract

In the previous issue of *Breast Cancer Research*, Gardner and co-workers describe a novel interaction between Geminin, a protein that prevents reinitiation of DNA replication, and Topoisomerase IIα (TopoIIα), an enzyme essential for removing catenated intertwines between sister chromatids. Geminin facilitates the action of TopoIIα, thereby promoting termination of DNA replication at the same time it inhibits initiation. In this manner, Geminin ensures that cells duplicate their genome once, but only once, each time they divide. Remarkably, either depletion of Geminin or over-expression of Geminin inhibits the action of TopoIIα, thereby making Geminin an excellent target for cancer chemotherapy.

The work of Gardner and colleagues [1] focuses the attention of breast cancer research on the small (23 to 25 kDa) protein Geminin, which is unique to multicellular animals. Geminin plays a pivotal role in coordinating DNA replication and cell division [2], as well as a role in specifying cell fate [3,4]. It accomplishes these varied tasks by inhibiting the activity of proteins involved in regulating genome duplication and gene expression [3,5-7]. The most well characterized example is Cdt1, one of eight proteins essential for loading the replicative DNA helicase (Mcm(2-7)) onto DNA replication origins. Geminin inhibition of Cdt1, however, is only one of five concerted pathways in metazoan cells that restrict nuclear DNA replication to one complete round per cell division, thereby maintaining genome stability and preventing cells from becoming aneuploid. However, since Geminin is selectively expressed in proliferating cells, and its level in cancer cells is markedly greater than in normal cells, it can be used as a biomarker for both the diagnosis and prognosis of cancer [8]. Moreover, suppression of Geminin has been suggested as a novel strategy for killing cancer cells with little or no effect on normal cells [9]. In contrast to normal cells, many cancer cells rely exclusively on Geminin to prevent reinitiation of DNA replication before mitosis is completed. Depletion of Geminin in these cells induces DNA re-replication, which results in accumulation of stalled replication forks and DNA damage. This, in turn, triggers apoptosis.

Working with human mammary epithelial (HME) cells, Gardner and colleagues [1] discovered that Geminin facilitates the ability of Topoisomerase IIα (TopoIIα) to bind chromatin and resolve catenated intertwines, and that this trimolecular interaction appears to be regulated by two protein kinases, one (CKIε) that activates TopoIIα, and one (Cdc7-Dbf4) that inhibits TopoIIα. Without TopoIIα activity, sister chromatids accumulate catenated intertwines during S-phase that prevent their separation during mitosis. This, in turn, triggers the spindle assembly checkpoint to arrest cells at metaphase and then induce apoptosis. Indeed, several chemotherapeutic drugs promote this reaction by inhibiting TopoIIα. HME cells treated with siRNA against Geminin also rapidly accumulate with 4N DNA content (G2 or M phase) and fail to complete cytokinesis due to chromosome bridges that remain between the two nuclei. This means that TopoIIα activity is suppressed during G1 phase when Geminin is absent and Cdc7-Dbf4 is present, but TopoIIα activity is facilitated from S through metaphase when Geminin is present. Thus, Geminin not only suppresses initiation of DNA replication, but also promotes termination of DNA replication forks.

There is, however, a significant difference between inhibiting TopoIIα binding to chromatin by suppressing Geminin and chemical inhibition of TopoIIα activity. Chemical inhibitors arrest cells in G2/M phase, but these cells soon by-pass the spindle assembly checkpoint and attempt to replicate their genome, a phenomenon termed 'mitotic slippage' [10]. Mitotic slippage occurs most frequently in cancer cells that lack p53 and Rb, components of checkpoints that prevent premature entrance into S phase. In contrast, siRNA depletion of Geminin in synchronized HME cells also suppressed expression of cyclins E and A1, Cdk1 and Cdk2, which would prevent initiation of DNA replication [11].

*Correspondence: depamphm@mail.nih.gov
National Institute of Child Health and Human Development, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892-2753, USA

However, this was not observed when Geminin was depleted in ten other cell lines derived from normal human tissues and three from cancer tissues [9]. These cells continued to proliferate normally and re-replicated their DNA only when both Geminin and cyclin A1 were suppressed. Cdk2-cyclin A1 is required in three of the five pathways that prevent DNA re-replication. *In vivo*, Geminin is largely dispensable for embryonic and adult mammalian neurogenesis [12], and it is not required for self-renewal of hematopoietic stem cells or baseline production of granulocytes or monocytes [13]. Thus, Geminin depletion is a promising therapy for killing cancer cells without interfering with normal cell proliferation.

Remarkably, over-expression of Geminin in HME cells triggers DNA re-replication (production of cells with >4N DNA). Under these conditions, TopoIIα cleaves the DNA without resealing the duplex and then dissociates from chromatin, leaving behind damaged DNA. However, since Geminin over-expression in HME cells is accompanied by suppression of both CHK1 and H2AX (components of the DNA damage response mechanism), and up-regulation of cyclin A1 and Cdk1 expression, this allows these cells to re-replicate their DNA and become aneuploid. Since TopoIIα is not associated with chromatin under these conditions, cells that over-express Geminin will be less sensitive to TopoIIα inhibitors that rely on trapping the TopoIIα-DNA adduct at the site of TopoIIα cleavage. Thus, the natural tendency of cancer cells to over-express Geminin may facilitate their ability to undergo chromosomal rearrangements and to resist the effects of TopoIIα inhibitors. Perhaps the high percentage of patients who do not respond to chemotherapeutic inhibitors of TopoIIα would respond if TopoIIα inhibitors were combined with anti-Geminin agents.

The effects of altering Geminin levels appear to depend on the cell and its genotype. Geminin depletion induces DNA re-replication in most, but not all, cancer cells [1,9]. Conversely, Geminin depletion does not arrest proliferation of non-cancer cells *in vitro* [9], nor does ablation of the Geminin gene prevent proliferation of all cell types *in vivo* [12,13]. However, some non-cancer breast cells may arrest in mitosis without inducing DNA re-replication or apoptosis [11]. Over-expression of a nondegradable form of Geminin in primary human fibroblasts arrests them in G1 without apoptosis, whereas over-expression in osteosarcoma cells induces apoptosis [14]. The fact that osteosarcoma cells expressing both p53 and Rb arrest in early S phase, whereas osteosarcoma cells that lack these genes accumulate in late S and G2/M, suggests that normal cells contain an 'origin licensing checkpoint' that prevents premature entrance into S phase [15], a hypothesis also supported by suppression of origin licensing proteins [16]. Cancer cells that lack this checkpoint would be vulnerable to drugs that increase Geminin activity. Thus, the ability to selectively kill cancer cells by either depletion or over-expression of Geminin bodes well for Geminin-based chemotherapies, but it remains to be determined through live animal studies just how useful such therapies will be.

Abbreviations

HME, human mammary epithelial; siRNA, small interfering RNA; TopoIIα, Topoisomerase IIα.

Competing interests

MLD has a patent pending on selective killing of cancer cells by suppression of geminin.

Published: 1 June 2011

References

1. Gardner L, Malik R, Shimizu Y, Mullins N, ElShamy WM: **Geminin overexpression prevents the completion of topoisomerase IIα chromosome decatenation leading to aneuploidy in human mammary epithelial cells.** *Breast Cancer Res* 2011, **13:**R53.
2. DePamphilis ML: *DNA Replication and Human Disease.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 2006.
3. Lim JW, Hummert P, Mills JC, Kroll KL: **Geminin cooperates with Polycomb to restrain multi-lineage commitment in the early embryo.** *Development* 2011, **138:**33-44.
4. Yellajoshyula D, Patterson ES, Elitt MS, Kroll KL: **Geminin promotes neural fate acquisition of embryonic stem cells by maintaining chromatin in an accessible and hyperacetylated state.** *Proc Natl Acad Sci U S A* 2011, **108:**3294-3299.
5. Miotto B, Struhl K: **HBO1 histone acetylase activity is essential for DNA replication licensing and inhibited by Geminin.** *Mol Cell* 2010, **37:**57-66.
6. Falaschi A, Abdurashidova G, Biamonti G: **DNA replication, development and cancer: a homeotic connection?** *Crit Rev Biochem Mol Biol* 2010, **45:**14-22.
7. Lutzmann M, Mechali M: **MCM9 binds Cdt1 and is required for the assembly of prereplication complexes.** *Mol Cell* 2008, **31:**190-200.
8. Haruki T, Shomori K, Hamamoto Y, Taniguchi Y, Nakamura H, Ito H: **Geminin expression in small lung adenocarcinomas: implication of prognostic significance.** *Lung Cancer* 2011, **71:**356-362.
9. Zhu W, Depamphilis ML: **Selective killing of cancer cells by suppression of geminin activity.** *Cancer Res* 2009, **69:**4870-4877.
10. Lee J, Kim JA, Margolis RL, Fotedar R: **Substrate degradation by the anaphase promoting complex occurs during mitotic slippage.** *Cell Cycle* 2010, **9:**1792-1801.
11. Nakuci E, Xu M, Pujana MA, Valls J, Elshamy WM: **Geminin is bound to chromatin in G2/M phase to promote proper cytokinesis.** *Int J Biochem Cell Biol* 2006, **38:**1207-1220.
12. Schultz KM, Banisadr G, Lastra RO, McGuire T, Kessler JA, Miller RJ, McGarry TJ: **Geminin-deficient neural stem cells exhibit normal cell division and normal neurogenesis.** *PLoS One* 2011, **6:**e17736.
13. Shinnick KM, Eklund EA, McGarry TJ: **Geminin deletion from hematopoietic cells causes anemia and thrombocytosis in mice.** *J Clin Invest* 2010, **120:**4303-4315.
14. Shreeram S, Sparks A, Lane DP, Blow JJ: **Cell type-specific responses of human cells to inhibition of replication licensing.** *Oncogene* 2002, **21:**6624-6632.
15. Blow JJ, Gillespie PJ: **Replication licensing and cancer - a fatal entanglement?** *Nat Rev Cancer* 2008, **8:**799-806.
16. Nevis KR, Cordeiro-Stone M, Cook JG: **Origin licensing and p53 status regulate Cdk2 activity during G(1).** *Cell Cycle* 2009, **8:**1952-1963.

doi:10.1186/bcr2881
Cite this article as: DePamphilis ML: **Spotlight on Geminin.** *Breast Cancer Research* 2011, **13:**109.