

EDITORIAL

Spotlight on Geminin

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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 II α (TopoII α), an enzyme essential for removing catenated intertwinings between sister chromatids. Geminin facilitates the action of TopoII α , 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 TopoII α , 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 II α (TopoII α) to bind chromatin and resolve catenated intertwinings, and that this trimolecular interaction appears to be regulated by two protein kinases, one (CKI ϵ) that activates TopoII α , and one (Cdc7-Dbp4) that inhibits TopoII α . Without TopoII α activity, sister chromatids accumulate catenated intertwinings 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 TopoII α . 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 TopoII α activity is suppressed during G1 phase when Geminin is absent and Cdc7-Dbp4 is present, but TopoII α 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 TopoII α binding to chromatin by suppressing Geminin and chemical inhibition of TopoII α 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].

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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, TopoII α 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 TopoII α is not associated with chromatin under these conditions, cells that over-express Geminin will be less sensitive to TopoII α inhibitors that rely on trapping the TopoII α -DNA adduct at the site of TopoII α 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 TopoII α inhibitors. Perhaps the high percentage of patients who do not respond to chemotherapeutic inhibitors of TopoII α would respond if TopoII α 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; TopoII α , Topoisomerase II α .

Competing interests

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

Published: 1 June 2011

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doi:10.1186/bcr2881

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