Cell Cycle 12:2, 191–192; January 15, 2013; © 2013 Landes Bioscience

"Take it up a NOTCH"
Novel strategies for cancer therapy

Stefanie L. Morgan, Gregory A. Wyant and Daniela M. Dinulescu*

Department of Pathology; Brigham and Women's Hospital; Harvard Medical School; Boston, MA USA

Abbreviations: CSCs, cancer stem cells; SP, side population; GSI, γ-secretase inhibitor; CDDP, cisplatin; GEMM, genetically engineered mouse models

Long-term survival of ovarian cancer patients is poor due to lack of early detection and high rates of therapeutic failure and chemoresistance. It is estimated that 15,500 women in the United States will die of ovarian cancer this year, and that nearly 23,000 more will receive a first-time diagnosis, 67% of whom will present with advanced stage disease (stages III and IV).[1,2] Only 5,200 of those are expected to survive more than 5 y due to the markedly few alternative therapies currently available for platinum-resistant tumors.[2,3] While the mechanism underlying platinum chemoresistance in ovarian cancer is extremely complex, drug-resistant cancer stem cells (CSCs), which evade the systemic effects of standard chemotherapies, are a major contributor to platinum-resistant disease.[4-7] Consequently, a cure likely necessitates the targeting and sensitization of CSCs in newly diagnosed tumors and makes the identification of key pathways for CSC function a necessity in present day cancer research.[5]

We recently developed a functional and molecular profile of ovarian CSCs to identify both stem cell surface markers and key pathways critical for CSC function.[7] It is important to note, however, that none of the markers we identified, including CD44, CD24, CD133 and ALDH1, appear to be completely specific for the isolation of ovarian CSCs.[7] In contrast, we found the side population (SP) phenotype to be an extremely reliable marker for the identification and characterization of tumor cells enriched for CSCs in a large number of experiments involving both murine and human samples.[7] Multiple studies have demonstrated that SP cells can be sorted from tumors based on their ability to efflux the Hoechst dye.[4,7] However, the accuracy of this method has been questioned at times, based on the incorrect assumption that the Hoechst dye may alter the cell cycle. Contrary to this assumption, our analysis of stained and unstained cells using the Modfit LT software demonstrated no difference between the cell cycles of the two populations, indicating that the optimized Hoechst staining is in fact a useful tool for the isolation and characterization of CSCs.

In characterizing the CSC profile, we have further established that Notch signaling, which has been implicated in the maintenance of tissue homeostasis by regulating self-renewal of stem cells and differentiation of progenitor cells, is a key pathway for CSC function. Dysregulation of Notch signaling can lead to transformation of normal stem cells and/or acquisition of self-renewing ability in early progenitor cells during tumorigenesis. Within the Notch pathway, Notch 3 amplification has been correlated with tumor recurrence, chemoresistance to carboplatin and a poor prognostic outcome.[6-8] Some data even suggest that activation of the Notch 3 pathway may reprogram tumor cells to assume a stem cell-like profile and contribute to platinum chemoresistance in ovarian cancer.[6,7] Thus, we have recently shown that overexpression of a constitutively activated Notch 3 receptor expands the number of CSCs and increases chemoresistance to platinum compounds.[7] In contrast, inhibition of Notch 3 activity using either siRNAs or γ-secretase inhibitors (GSI) sensitizes ovarian cancer cells to platinum-based therapies.[6,7] Furthermore, our previous findings have indicated that a dual combination of GSI and cisplatin (CDDP) therapy, which targets both CSCs and non-CSCs, is a much more effective treatment than CDDP alone (**Fig. 1**), for both platinum-sensitive and -resistant patient samples.[7]

However, as this increased sensitivity is only observed in tumors with Notch 3 overexpression and activation of the Notch pathway, the use of genetic biomarkers to identify which patients are most likely to benefit from GSI-based therapy is critical. Beyond patient screening, a second critical element for successful clinical trials is the use of functionally similar compounds in both research and clinic. This is of particular concern with GSIs, as there are over 20 different compounds available for research and clinical use, the efficacy and toxicity of which varies greatly. Of additional concern is the use of GSI monotherapy as a treatment strategy in clinical trials. Although GSI is effective in sensitizing cells to cytotoxic agents, such as platinum compounds, it does not contribute to long-term remission when used alone.[7] Our data further suggest that Notch inhibitors should be used as a first line of treatment in combination with platinum in order to achieve the best results therapeutically.[7] Finally, it is important to identify a Notch-based signature to better assess drug responses in clinical trials. Failure to address these guidelines likely played a significant role in the lack of success of two recently

*Correspondence to: Daniela M. Dinulescu; Email: ddinulescu@rics.bwh.harvard.edu
Submitted: 11/15/12; Accepted: 11/21/12
http://dx.doi.org/10.4161/cc.23375
Comment on: McAuliffe SM, et al. Proc Natl Acad Sci USA 2012; 109:E2939-48; PMID:23019585; http://dx.doi.org/10.1073/pnas.1206400109

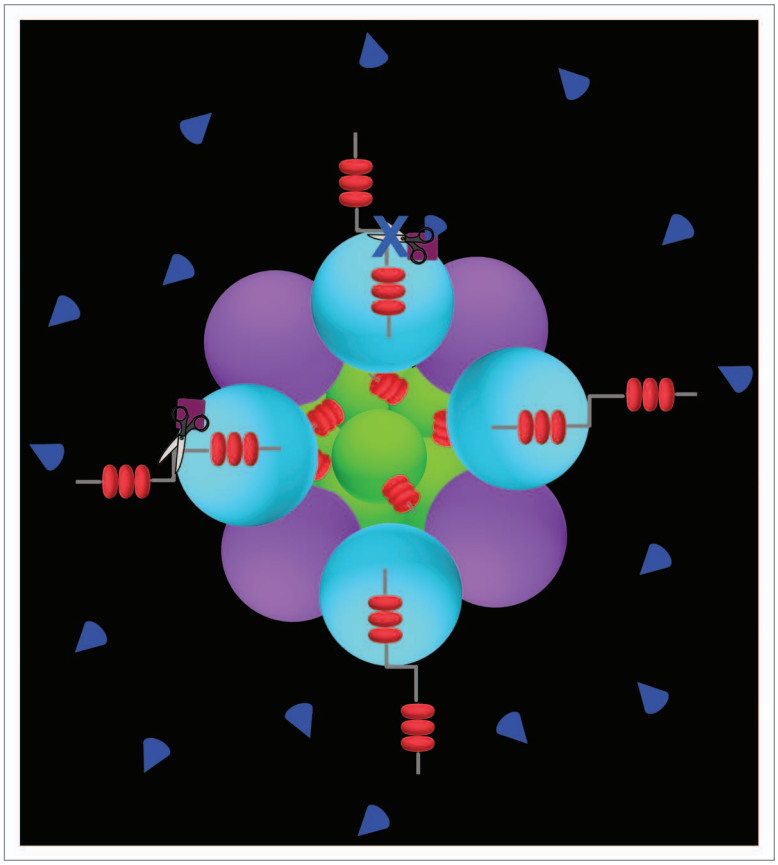

Figure 1. Cancer stem cells (CSCs) are key contributors to tumor chemoresistance due to their ability to survive platinum-based chemotherapies. Pictured is a graphical representation of a Notch-dependent ovarian tumor that accumulates drug-resistant CSCs (green circles) and acquires platinum resistance. Differentiated Notch-dependent non-CSC tumor cells are shown in light blue circles. McAuliffe et al.[7] shows that tumor sensitivity to platinum is restored when the γ-secretase complex (plum), which cleaves and activates the Notch receptor (red), is targeted with γ-secretase inhibitor I (GSI, navy blue triangles).

completed clinical trials using GSIs, which resulted in one GSI compound being discontinued indefinitely.[9] Consequently, it is important that clinical trials replicate the design of successful preclinical studies whenever possible.

A further challenge to the clinical application of GSIs has been their gastrointestinal toxicity and off-target effects.[9] In an attempt to remedy this issue, inhibitory antibodies have recently been synthesized for all Notch receptors, including Notch 3.[10] This will pave the way for new clinical trials to evaluate the efficacy of more selective and less toxic antibody-based therapies in enhancing the response to platinum treatment. The overwhelming potential of Notch-based cancer treatments cannot be ignored. Increasing the use of personalized tumor biomarkers and translating these novel therapies into practice hold great promise for achieving a better prognosis in ovarian cancer.

Author Contributions

S.L.M. and D.M.D. designed research, S.L.M. and G.A.W. performed research; S.L.M., G.A.W. and D.M.D. analyzed data; S.L.M. and D.M.D. wrote the paper.

References

1. Siegel R, et al. CA Cancer J Clin 2012; 62:10-29; PMID:22237781; http://dx.doi.org/10.3322/caac.20138
2. Kosary CL. 2007; in: Ries LAG, Young JL, Keel GE, et al., *SEER Survival Monograph*. Cancer Survival Among Adults: U.S. SEER Program, 1988-2001 Patient and Tumor Characteristics. National Cancer Institute, SEER Program, NIH Pub. No. 07-6215, Bethesda, MD
3. Trouskova O, et al. Adv NPs PAs 2012; 3:21-4, quiz 25; PMID:22355885
4. Szotek PP, et al. Proc Natl Acad Sci USA 2006; 103:11154-9; PMID:16849428; http://dx.doi.org/10.1073/pnas.0603672103
5. Gupta PB, et al. Cell 2009; 138:645-59; PMID:19682730; http://dx.doi.org/10.1016/j.cell.2009.06.034
6. Park JT, et al. Am J Pathol 2010; 177:1087-94; PMID:20671266; http://dx.doi.org/10.2353/ajpath.2010.100316
7. McAuliffe SM, et al. Proc Natl Acad Sci USA 2012; 109:E2939-48; PMID:23019585; http://dx.doi.org/10.1073/pnas.1206400109
8. Cancer Genome Atlas Research Network. Nature 2011; 474:609-15; PMID:21720365; http://dx.doi.org/10.1038/nature10166
9. Gounder MM, et al. J Clin Oncol 2012; 30:2291-3; PMID:22564999; http://dx.doi.org/10.1200/JCO.2012.42.3277
10. Wu Y, et al. Nature 2010; 464:1052-7; PMID:20393564; http://dx.doi.org/10.1038/nature08878
11. McAuliffe SM, et al. Proc Natl Acad Sci USA 2012; 109:E2939-48; PMID:23019585; http://dx.doi.org/10.1073/pnas.1206400109