Beyond Genetics: Surprising Determinants of Cell Fate in Antitumor Drugs

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In this issue of *Cancer Cell*, Gascoigne and Taylor (2008) report their findings of following 10,000 single cells incubated with three classes of antimitotic drugs, including paclitaxel (taxol). This extensive analysis reveals a previously unappreciated complexity in response to such drugs and demonstrates that it is more than genetics that determines cell life or death.

To maintain its chromosome content, each dividing cell must partition its replicated genome such that one copy of each chromosome is deposited into each new daughter cell. Errors in this process lead to the production of aneuploid daughter cells that contain an abnormal chromosome content. Aneuploidy can have drastic consequences for both the cell and the organism (Kops et al., 2005). Indeed, while most aneuploid human embryos are inviable, those that do survive develop severe birth defects such as Down's syndrome (Jacobs and Hassold, 1995). Furthermore, aneuploidy is a common characteristic of tumor cells and has been shown in some instances to play a causative role in the development of cancer (Weaver et al., 2007).

A major safeguard for protecting cells against chromosome missegregation is the mitotic checkpoint (also known as the spindle assembly checkpoint) (Kops et al., 2005; Musacchio and Salmon, 2007). This cell-cycle control mechanism operates in every mitosis to ensure the faithful partitioning of the duplicated chromosomes between the two daughter cells. Chromosomes are sorted in mitosis by the microtubule spindle, to which they attach at a specialized protein structure known as the kinetochore. Unattached kinetochores are the source of the mitotic checkpoint signal and act by producing an inhibitor that delays cyclin B1 degradation and chromosome segregation until all chromosomes have stably attached to the spindle.

Some drugs that target the mitotic checkpoint have a long history as successful anticancer agents and are commonly used in the treatment of a variety of human cancers (Jackson et al., 2007). These agents, known as antimitotic drugs, generally act by chronically activating the mitotic checkpoint, leading to a prolonged mitotic delay (Rieder and Maiato, 2004). There are several possible outcomes of this drug-induced mitotic delay. Cells may undergo death while arrested in mitosis or exit mitosis in the presence of the drug and transition into the subsequent interphase, a process known as adaptation. Filming of cyclin B1 levels in living cells has suggested that cyclin B1 is progressively lost during a mitotic delay (Brito and Rieder, 2006). Eventually, cyclin B1 levels fall below the threshold required to maintain mitotic arrest and adaptation occurs. Adapted cells may then continue to cycle, senesce, or execute death pathways from interphase. Despite the clinical importance of predicting which tumors will show favorable responses to antimitotic drugs, the pathways that determine whether a cell dies, arrests, or proliferates following treatment with antimitotic drugs are not known (Rieder and Maiato, 2004).

Determining Cell Fate

In this issue, Gascoigne and Taylor (2008) provide the most in-depth analysis to date of the response of cancer cells to antimitotic agents. Utilizing high-throughput automated time-lapse microscopy, the authors determined the response of individual cells from a large panel of tumor cell lines treated with three classes of antimitotic drugs. As anticipated from earlier work, different tumor cell lines varied widely in their response to antimitotic agents. Surprisingly, however, cells within the same line often also displayed substantial variation in their response to drug treatment. The authors elegantly and persuasively demonstrate that the variation in the fate of individual cells within a population cannot be accounted for by initial genetic variation. Indeed, even presumably genetically identical daughter cells frequently behave differently in the next mitosis, varying in their response as much as two cells chosen at random from the population. Thus, cell fate in response to chronic mitotic checkpoint activation is not genetically predetermined. Instead, Gascoigne and Taylor's observations implicate an unexpected level of stochastic variation in the signaling pathways activated by antimitotic agents.

Having demonstrated tremendous variation in the behavior of cells to antimitotic agents, Gascoigne and Taylor formulate and test a model in which mitotic cell fate is determined by two competing networks, one involving the slow destruction of cyclin B1, the other the progressive activation of a putative caspase-dependent death pathway. By establishing the threshold level of cyclin B1 that is required for mitotic exit, the authors demonstrate that cells that typically die directly from mitosis only slowly degrade cyclin B1, thereby failing to breach the threshold required for mitotic exit prior to death (Figure 1B). By contrast, cells that undergo adaptation progressively destroy cyclin B1 and surpass this threshold, leading to mitotic exit (Figure 1A). For each cell line tested, inhibiting caspase activation with a pan-caspase inhibitor not only reduced the proportion of cells that died in mitosis but also prolonged the duration of the mitotic arrest. This suggests that by inhibiting caspase-dependent death signals produced by chronic

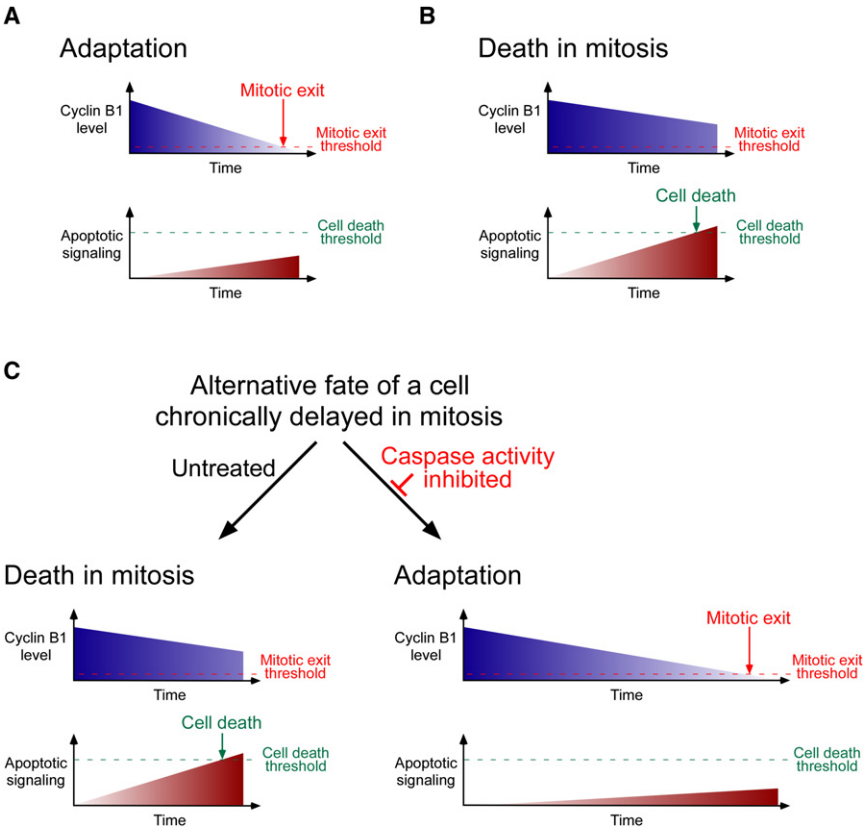

Figure 1. Model for Two Competing Networks that Together Dictate Mitotic Cell Fate
(A) Cyclin B1 (blue) is progressively destroyed during a mitotic delay, and at the same time, a putative death signal (red; possibly also produced by the mitotic checkpoint) accumulates. Eventually, cyclin B1 levels fall bellow a threshold required to maintain mitotic arrest, and the cell undergoes adaptation.
(B) Cyclin B1 levels fall during mitotic delay but fail to decline to the threshold required to allow mitotic exit. Instead, the apoptotic signal reaches a level sufficient to promote cell death in mitosis.
(C) Slowing the activation of apoptotic effectors by treatment with a caspase inhibitor provides a cell with more time to destroy cyclin B1. This allows cyclin B1 levels to reach the threshold required to promote mitotic exit before apoptosis is initiated.

mitotic checkpoint signaling, cells are provided with more time to degrade cyclin B1 to a level sufficient to promote mitotic exit (Figure 1C).

Chromosomal Instability Revisited

Aneuploidy is a remarkably common characteristic of tumor cells often resulting from an underlying chromosomal instability (CIN), characterized by frequent gains and losses of chromosomes during cell division (Kops et al., 2005). A long-standing, widely held view is that chromosomally unstable cancer cells possess a weakened mitotic checkpoint that permits the onset of anaphase in the presence of misaligned chromosomes. This dogma was challenged in a recent study showing that CIN cells do not prematurely initiate anaphase but rather exhibit an increased incidence of lagging anaphase chromosomes (Thompson and Compton, 2008). Gascoigne and Taylor (2008) confirm these observations and further demonstrate that CIN cells arrest in mitosis for as long as chromosomally stable cell lines. Taking the results of these studies together, the evidence is now compelling that the mitotic checkpoint is not compromised in chromosomally unstable cancer cells. Moreover, the detailed single-cell analysis dispels the proposal that CIN cells are more sensitive to the killing action of antimitotic drugs.

Utilizing their extensive data set, Gascoigne and Taylor further explore the widely held view that the duration of mitotic delay may dictate the subsequent cellular response. Importantly, however, no correlation was found between length of delay and resulting cell fate. This concurs with a recent report showing that the ability of the mitotic checkpoint to delay cells in mitosis is a poor predictor of cellular response to antimitotic drugs (Shi et al., 2008). Rather, cell fate was found to correlate much more closely with the sensitivity of cell lines to apoptosis induced by other factors, such as the broad-spectrum kinase inhibitor staurosporine (Shi et al., 2008). This implies that sensitivity to apoptotic signaling may be an important source of variation in the response of cancer cells to antimitotic drugs and suggests that more favorable drug responses may be achieved by combining antimitotic drugs with agents that restore apoptotic pathways in death-resistant cells.

Future Directions

Previous studies investigating cell fate in response to antimitotic agents have predominantly relied upon indirect assays that average the responses of populations of cells. The enhanced resolution afforded by direct single-cell assays has now revealed that these prior studies have dramatically underestimated the variation in cell fate in response to antimitotic drugs. Central questions now raised, but still left unanswered, include: What is the identity of the putative death signal that accumulates during a mitotic delay? What are the relative thresholds of cyclin B1 and apoptotic signaling required to promote adaptation and death in various cell lines? What are the pathways responsible for determining cell fate following adaptation, and why do some cell lines show dramatically different responses to different antimitotic drugs that all chronically activate the mitotic checkpoint? Finally, it is important to bear in mind that the in vitro conditions used here fail to recapitulate aspects of the tumor microenvironment that may have important influences on cell fate including, for example, drug clearance by the circulation, which would lead to renewed cell cycling. Indeed, knowing that stochastic processes can be central to the cell's decision to live or die leaves open the question as to how closely the response of cell lines will model the fate of a heterogeneous population of tumor cells.

REFERENCES

Brito, D.A., and Rieder, C.L. (2006). Curr. Biol. *16*, 1194–1200.

Gascoigne, K.E., and Taylor, S.S. (2008). Cancer Cell *14*, this issue, 111–122.

Jackson, J.R., Patrick, D.R., Dar, M.M., and Huang, P.S. (2007). Nat. Rev. Cancer *7*, 107–117.

Jacobs, P.A., and Hassold, T.J. (1995). Adv. Genet. *33*, 101–133.

Kops, G.J., Weaver, B.A., and Cleveland, D.W. (2005). Nat. Rev. Cancer *5*, 773–785.

Musacchio, A., and Salmon, E.D. (2007). Nat. Rev. Mol. Cell Biol. *8*, 379–393.

Rieder, C.L., and Maiato, H. (2004). Dev. Cell *7*, 637–651.

Shi, J., Orth, J.D., and Mitchison, T. (2008). Cancer Res. *68*, 3269–3276.

Thompson, S.L., and Compton, D.A. (2008). J. Cell Biol. *180*, 665–672.

Weaver, B.A., Silk, A.D., Montagna, C., Verdier-Pinard, P., and Cleveland, D.W. (2007). Cancer Cell *11*, 25–36.

Even Cancers Want Commitment: Lineage Identity and Medulloblastoma Formation

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In this issue of *Cancer Cell*, Yang et al. (2008) and Schüller et al. (2008) show that Hedgehog activation in either multipotent neural stem cells or developmentally restricted progenitors causes only medulloblastomas to form. These data suggest that some stem cell-derived tumors must commit to a specific lineage in order to grow.

The cellular origins of most solid tumors are not well understood, in part because specific markers for the stem, progenitor, and differentiated cell populations from which they might arise are often lacking. Medulloblastomas are central nervous system (CNS) embryonal tumors composed of primitive-appearing cells that can differentiate along multiple lineages (Louis et al., 2007). They arise in the cerebellum, but it is not yet clear whether multipotent stem cells, developmentally restricted progenitors, or other cells give rise to these pediatric malignancies. It is hoped that by defining the cell (or cells) in which they form and the relationship between normal development and oncogenesis, improved therapies can be developed.

The cerebellar ventricular zone (VZ) consists of a band of stem and progenitor cells that line the IVth ventricle of the cerebellum (Figure 1). It gives rise to most cell types in the cerebellum, including Bergmann glia, astrocytes, oligodendrocytes, and Purkinje neurons (Sillitoe and Joyner, 2007). At one edge of this zone lies the rhombic lip, which sends forth a stream of lineage-restricted granule neuron precursors (GNPs) over the cerebellar surface. These proliferate transiently in the external germinal/granular layer (EGL) and then migrate inward and differentiate to form the numerous small neurons of the internal granule cell layer (IGL).

It has become clear that many molecular pathways play similar roles in both cerebellar development and medulloblastoma formation. Hedgehog signaling, for example, drives proliferation of the cerebellar EGL and is also activated in both familial and sporadic medulloblastomas, most commonly by mutations or deletions abrogating function of the inhibitory receptor *Patched* (*Ptc*) (Louis et al., 2007; Pietsch et al., 1997). Transgenic mice lacking one copy of *Ptc* develop medulloblastomas that appear to arise from proliferations of GNP-like cells in the EGL (Kho et al., 2004; Oliver et al., 2005), suggesting that medulloblastomas form in lineage-committed progenitor cells. However, this notion must be reconciled with the fact that medulloblastomas are thought to differentiate into both glia and neurons, occasionally even forming melanocytic or muscle cells (Louis et al., 2007). While such a broad cellular spectrum could result from dedifferentiation of a committed GNP due to the influence of oncogenic molecular events, it is also possible that the tumors truly form in multipotent stem cells with an intrinsic multilineage potential. Indeed, several studies have suggested that some medulloblastomas express markers normally found in VZ stem cells and their descendants, but this is not proof that the tumors came from stem cells (Eberhart, 2007).

In this issue of *Cancer Cell*, Yang et al. (2008) and Schüller et al. (2008) directly address the issue of which CNS cells are capable of being transformed into tumors by Hedgehog activation. They find that while neoplasms can be "initiated" when genetic changes are induced in either multipotent VZ cells or lineage-committed cells, tumor masses appear to first form in the EGL and have a largely GNP-like phenotype.

Yang and colleagues (2008) use conditional *cre*-mediated deletion of *Ptc* in knockout mice ($Ptc^{C/C}$) to activate the Hedgehog pathway in either GNPs or multipotent stem cells. Activation in Math1-expressing GNPs of the rhombic lip and EGL resulted in a marked expansion and inappropriate persistence of this progenitor layer during early postnatal life. Many of these cells were able to exit the cell cycle,