Tumor Dormancy and MYC Inactivation: Pushing Cancer to the Brink of Normalcy

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Abstract

Upon MYC inactivation, tumors undergo proliferative arrest, cellular differentiation, apoptosis and in some cases, permanently revoke tumorigenesis. In liver tumor cells, we recently showed that MYC inactivation uncovers stem cell properties and triggers differentiation, but in this case, their neoplastic properties are restorable by MYC reactivation. Thus, whereas oncogene inactivation can push cancer to the brink of normalcy, some cells retain the latent capacity to turn cancerous again, arguing that they may exist in a state of tumor dormancy. (Cancer Res 2005; 65(11): 4471-4)

Introduction

Cancer is one of the most common causes of death. Since the identification of the first activated proto-oncogene, the targeted inactivation or repair of these mutant gene products has been studied as a potentially therapeutically useful approach for the treatment of neoplasia (1). To date, only a few drugs have been identified that have been successfully shown to have clinical efficacy through the inactivation of a specific oncogene (2, 3). However, recent work in experimental transgenic model systems suggests that the targeted inactivation of a single oncogene may be generally effective in the treatment of some cancers (4).

Transgenic mouse models provide a powerful approach to preclinically evaluate the mechanisms by which genetic events initiate and sustain tumorigenesis. The development of conditional transgenic model systems has provided the unique ability to directly interrogate when oncogene inactivation will be effective in inducing tumor regression (4, 5). A common general feature of oncogene inactivation in a wide spectrum of tumors arising in different cellular lineages is that upon oncogene inactivation tumors undergo proliferative arrest, differentiation, and/or apoptosis. Thus, oncogene inactivation in some cases seems able to reverse the neoplastic phenotype.

Surprisingly, under some circumstances, even the brief inactivation of a single oncogene can be sufficient to induce sustained tumor regression, strongly indicating that, at least in some cases, oncogene inactivation can cause permanent loss of the neoplastic phenotype, indicating that cancer has been “reverted” (6). These observations are consistent with clinical experience in human cancer patients, where disease can be cured in some cases by administration of a limited number of cycles of chemotherapy, radiation therapy, or other biological therapies. The observation that such treatments can ever be efficacious suggests that a therapeutic approach that transiently affects the biology of a cancer cell can be fully effective. In many cases, therapy results in the irreversible loss of a cancer phenotype through necrosis or apoptosis, but in other cases, it is clear that the cancer cells are still present and either that they no longer behave as cancer cells or that they can remain dormant for long periods of time.

Recent results from several laboratories suggest that MYC inactivation in some cancers will not revoke tumorigenesis; rather, it will induce a transient loss of neoplastic character, marked by normal phenotype, that can be fully restored by MYC reactivation (6–9). To develop successful new treatments for cancer, it is important to learn to distinguish when oncogene inactivation induces stable tumor regression associated with a permanent loss of a malignant phenotype, versus when oncogene activation merely results in a reversible state of tumor dormancy.

MYC Inactivation: Tumorigenesis Reverted versus Tumor Dormancy

The consequences of MYC inactivation and reactivation seem to depend upon the particular type of cancer. In MYC-induced lymphoma, MYC inactivation results in differentiation, robust apoptosis, and complete tumor regression (10). These tumors regress despite the complexity of the genomic events acquired by the tumors (11). Thus, MYC inactivation in lymphoma commonly results in a permanent loss of a neoplastic phenotype. In contrast, in MYC-induced osteogenic sarcoma, MYC inactivation induces differentiation and dramatically reduces the rate of cellular proliferation but does not induce significant apoptosis. In this in vivo setting, MYC inactivation seems to lead to the return of normal bone morphology function (6). Reactivation of MYC in these apparently differentiated tumor cells either has no consequence or induces apoptosis; only very rare cells restore the operation of neoplastic pathways. These studies show that MYC inactivation is able to both reverse and revoke a neoplastic phenotype.

In contrast, in MYC-induced hepatocellular carcinoma, MYC inactivation initially induces differentiation and only later is followed by gradual apoptosis of the majority of the tumor cells. The differentiation process seems to uncover stem cell properties because after MYC inactivation, the cells are able to differentiate into multiple cell lineages that form structures characteristic of normal hepatocytes and biliary cells (7). However, in this setting, reactivation of MYC causes these differentiated tumor cells to rapidly become tumorigenic. We have suggested the following model to interpret these results (Fig. 1). MYC inactivation uncovers the stem cell properties of liver tumor cells, permitting them to differentiate into normal liver cell lineages; however, some of these normal-appearing cells actually remain in a latent state, such that upon MYC reactivation they rapidly restore their neoplastic features. As described below, we propose that this

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happens because some of the differentiated tumor cells are cancer stem cells that can regain their neoplastic properties. In this fashion, MYC inactivation induces a state of tumor dormancy.

We performed a variety of experiments to rule out the possibility that tumors that reemerged after MYC reactivation were derived from hepatocytes that were initially only partially malignantly transformed. We found that normal hepatocytes from the MYC transgenic mouse model used could not persist when transplanted into new hosts, even when coinoculated with tumor cells. We also did this test using liver tumor cells from a lung metastasis, obtaining identical results, arguing against the remote possibility that normal hepatocytes that may be present in a primary tumor had metastasized along with cancer cells to the lung. Finally, using array comparative genomic hybridization, we confirmed that the parental tumor, transplanted tumor, dormant tumor, and restored tumor contained an identical genetic signature, providing very strong evidence that they were derived from the same clone.

Another consideration was whether the liver tumors that recurred upon MYC reactivation were derived from normal-appearing differentiated tumor cells or from rare cancer cells that were undetectable by histologic analysis. These rare cells, whereas quiescent, may have failed to undergo differentiation because they had acquired some additional genetic hits that allowed them to become independent of MYC. Two lines of evidence argued against this explanation for tumor recurrence. First, using bioluminescence imaging, we were able to do highly quantitative measurements on the rate of tumor regression and reemergence. Based upon the signal intensity, it seemed that a large fraction of the tumor cells were proliferatively expanding upon MYC reactivation. Second, in the tumors that returned upon MYC reactivation, a second round of MYC inactivation induced tumor regression identically to the parent tumor. This observation argued against the likelihood that secondary genetic events acquired during initial tumor formation were responsible for compromising sensitivity to MYC inactivation.

Several other recently published results have also described that oncogene inactivation can induce tumor regression and that oncogene reactivation leads to rapid tumor reformation. Thus, MYC-induced skin and pancreatic islet cell tumors undergo regression upon MYC inactivation and that tumors reform quickly after MYC reactivation (8). Similarly, MYC-induced breast cancer undergoes regression upon MYC inactivation that is fully revoked by MYC reactivation (12). In RAS-induced melanoma, oncogene inactivation similarly induces tumor regression; however, in this case, residual microscopically detectable cancers remained that rapidly reemerged as large tumors upon RAS reactivation (13). Similarly, in Gli-induced skin tumors, residual disease remaining after oncogene inactivation reemerged rapidly upon Gli reactivation (14). Thus, in many cases, oncogene inactivation does not result in complete and sustained tumor regression (with the caveat that in some cases there might have been a failure to achieve full inactivation of the tumor-driving oncogene).

Taken together, MYC transgenic mouse studies suggest that there are circumstances when inactivation of the MYC oncogene can induce a state of tumor dormancy. These results have potential implications for the treatment of cancer discussed further below.

**Tumor Dormancy and Cancer Stem Cells**

Tumor dormancy, observed clinically, is generally thought to reflect the existence of residual tumor cells that fail to respond to a...
conventional treatment regimen (15–18). Residual tumor cells may be kept in check by various mechanisms, including the host immune system, tumor microenvironment, and/or angiogenic or autocrine growth factor capability (19–23). Our findings are not exclusive of these possibilities. The notion that cancer can be "reprogrammed" is not new and has been recently validated through Herculean efforts that illustrate how normal mice can be generated, albeit rarely, via nuclear transfer from a cancer cell (24, 25).

How might oncogene inactivation allow tumor cells to uncover latent stem cell–like properties? One possibility is that tumors, even if they seem morphologically or histologically homogenous, consist of different cell populations with discrete differences at the molecular level. This is consistent with the evidence from other groups indicating that cancer might originate from the transformation of a cancer stem cell. In leukemia and breast cancer, a specific subgroup of the tumor is able to propagate the tumorigenic properties of the cancer. If liver cancer induced by MYC activation arises specifically from stem cells, then upon MYC inactivation one might predict that these stem cells would regain the ability to resume their physiologic program and to undergo differentiation into progenitor cells as well as more mature cell lineages. However, because of their stem cell roots, these differentiated cells might be capable of maintaining a population of cells that can self-renew at a slower rate. In these cells, which retain some stem cell features, reactivation of the MYC oncogene could result in the restoration of the neoplastic program.

Still, other possibilities can be envisaged. For example, MYC overexpression may simply usurp or defeat certain normal programs of cell fate. MYC plays important roles in embryonic and organ development; thus, it is conceivable that the programs affected by MYC are shared with programs found in stem cells. Upon MYC inactivation, these programs would be released from the domination of MYC, such that the normal program can resume, similar to what occurs during normal organ development or regeneration in the stem cell compartment. Two other possibilities are that different cell populations are transformed by MYC, such that MYC inactivation releases the different populations to their normal cellular programs, resulting in different cell lineages; or that a normal cell fuses with a tumor cell in the primary tumor, somehow leading the heterokaryon to exhibit stem cell properties. These possibilities seem to offer poor explanations of the response of hepatocytes to cycles of MYC activation and inactivation, because the molecular mapping of the tumors showed that the tumors are clonal even when MYC has been inactivated and reactivated for several rounds, including the domination of MYC, such that MYC inactivation releases the different populations to their normal cellular programs, resulting in different cell lineages; or that a normal cell fuses with a tumor cell in the primary tumor, somehow leading the heterokaryon to exhibit stem cell properties. These possibilities seem to offer poor explanations of the response of hepatocytes to cycles of MYC activation and inactivation, because the molecular mapping of the tumors showed that the tumors are clonal even when MYC has been inactivated and reactivated for several rounds, including the following several series of transplantation.

Clinical and Therapeutic Implications of Tumor Dormancy

Our results suggest that targeted therapy may induce tumor regression and an apparent clinical elimination of the malignancy by histologic criteria. There may be instances when what has actually occurred is restoration of normal tissues that include a population of occult and dormant tumor cells. Thus, a determination that a cancer has been truly eliminated may sometimes require more than a measurement of the size of a tumor or an examination of the tissue histology to rule out the presence of dormant tumor cells that nevertheless appear and behave normally.

The notion that cancer can become dormant upon treatment is supported by decades of clinical experience (26). Treatment of many types of cancers, including breast cancer, lymphoma, leukemia, and sarcomas, can be associated with long-term regression of tumors, only to be followed by relapse at a much later time (15–18). The specific type of cancer can often predict the outcome of treatment. Thus, hematopoietic cancers are perhaps one of the best examples of how treatment outcome is influenced by the specific type of tumor. High-grade lymphomas like Burkitt’s lymphoma and diffuse large cell lymphoma frequently respond to chemotherapy with rapid apoptosis. In these cases, cancer is readily cured. In contrast, low-grade lymphomas, such as follicular lymphoma, whereas also responding to therapy, are rarely seen to regress completely (27).

The recent findings of transgenic mouse studies may offer explanations of what has been seen for many years in the clinic. Some types of cancer exhibit sustained regression to treatment and remain in remission. Others will undergo initial regression but remain present in an occult dormant state that reemerges upon cessation of therapy, leading to a rapid reoccurrence. Clinical outcome will no doubt reflect the biology unique to specific subtypes of cancer and their underlying features. A successful therapeutic strategy for cancer will therefore require an understanding of the nature of how genetic events and epigenetic context of a tumor can be used to anticipate consequences of gene product inactivation, such that one can achieve a complete elimination of tumor cells or a permanent reversal of tumorigenesis.

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sustained tumor regression upon MYC inactivation unless they acquire novel chromosomal translocations.


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