Oncogene addiction: Sometimes a temporary slavery

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Tumors induced in conditional oncomice can show remarkable different responses to subsequent oncogene deprivation. Complete sustained regression, concomitant with massive differentiation and/or apoptosis, and partial regression are both observed. In the latter case, tumor growth either resumes without being dependent any longer on the oncogene, or requires reactivation of the oncogene in cells that have become dormant. These models reflect many of the features we also witness in human cancer and can therefore assist us in understanding the underlying mechanisms and in designing more effective treatment protocols.

Some tumors show a surprisingly tight dependence on the continued activity of a specific oncogene, even in the presence of additional tumorigenic lesions. This is best illustrated in chronic myeloid leukemia in man, where imatinib mesylate (Gleevec) can cause complete regression of advanced tumors, already refractory to regular cytotoxic drugs, by specifically inhibiting the tyrosine kinase activity of the overexpressed BCR-ABL oncoprotein. The dependence of this tumor on expression of BCR-ABL is most convincingly illustrated by the observation that in recurrent disease, the BCR-ABL often has undergone point mutations that make the kinase refractory to the drug (Shah and Sawyers, 2003). The obvious strategy then is to treat CML with multiple drugs that inhibit the different conformational variants of the BCR-ABL oncoprotein. In the case of imatinib treatment, there are very few side effects, indicating that inhibition of physiological levels of ABL in normal cells does not severely affect their functionality. Therefore, only the tumor is addicted to high expression of ABL, and cannot survive without it. This "addiction," likely fostered by collaborating mutations present in the tumor cells, provides an unanticipated enhancement of the therapeutic index of the drug. One wonders whether this principle might be also valid in other settings.

A number of conditional mouse models have been recently described that address this oncogene addiction paradigm (Weinstein, 2002). Most revealing are the models that activate the same oncogene in a range of different tissues. We will focus in this minireview on conditional Myc models, as they constitute an intriguing set to compare (Table 1). Induction of the Myc oncogene in the suprabasal layer of skin resulted in massive hyperplasia and papillomatosis with extensive angiogenesis in the dermis. Complete regression ensues after downregulation of Myc (Pelengaris et al., 1999). Interestingly, Myc overexpression appeared to suffice to drive this complex process of papillomatosis without the apparent requirement for complementing mutations. Although this situation is rather artificial, as all suprabasal cells are forced to express high levels of Myc and therefore might escape from the growth-suppressive signals of normal neighboring cells, it is remarkable that Myc overexpression alone can catalyze such complex processes. The skin appears to respond to Myc without a requirement for additional complementing mutations that suppress apoptosis, a genetic alteration many cell types need to tolerate overexpression of Myc. The tight dependence on continued expression of Myc is not unique for the skin tumor model. In hematopoietic tumors induced by overexpression of Myc, a similar dependence is seen. There, Myc downregulation results in differentiation and apoptosis of tumor cells and resumption of normal hematopoiesis (Felsher and Bishop, 1999). Overexpression of Myc in pancreatic islets, however, causes degeneration of the islets by apoptosis. Clearly in this case, the increased sensitivity to apoptosis conferred by Myc prevails over its proliferative effect with the net result of pancreatic β cell ablation. When Myc expression is induced in islets that overexpress the antiapoptotic BclXL oncogene, the apoptotic effect of Myc is rescued, and massive invasive tumors ensue (Pelengaris et al., 2002). Interestingly, downregulation of Myc reverses this condition to normal, emphasizing the dominance and dependence of Myc overexpression for the maintenance of this tumor. An even more astonishing observation was recently made in Myc-induced osteogenic sarcomas. Brief downregulation of Myc resulted in differentiation of the cells into mature bone. Subsequent reexpression of Myc did not reinitiate tumor growth, but rather caused apoptosis, indicating that these differentiated cells could not regain tumorigenic properties (Jain et al., 2002). This might be explained by the incapacity of differentiated cells to dedifferentiate and reestablish tumorigenic growth. It also indicates that, at least in this instance, no progenitor cells have survived that could reinitiate tumor growth. This raised the intriguing possibility that temporary downregulation of aberrantly expressed oncogenes by small molecules or RNAi inhibition might cause long-lasting tumor regression. This perspective is particularly attractive in view of the fact that many of the specific inhibitors of oncogenic pathways can have substantial side effects.

Two recent Myc-based tumor models show again a different situation. In the first model, Myc overexpression in hepatocytes results in hepatocellular carcinomas or hepatoblastomas (Shachaf et al., 2004). Subsequent downregulation of Myc caused tumor regression with concomitant differentiation and apoptosis. However, a portion of the cells survive and remain dormant. These cells can persist for long periods and show features of cancer stem cells (Pardal et al., 2003). The survival of these cells apparently requires the temporary expansion conferred by Myc and probably involves additional mutations that mediate a survival advantage to these cells. This is underscored by the presence of distinct chromosomal aberration in these

Tumor development, regression, and relapse in conditional MYC tumor models

Model	Conditional system	Tumor phenotype	Latency	Neoplastic progression	Clonality	Initial response to oncogene inactivation	Mechanism of regression	Relapse	Mechanism of relapse	Reference
INV-MycER^T	Tamoxifen	Papilloma	3 weeks	Ubiquitous	ND	Regression	Cell cycle arrest, differentiation, vascular degeneration	ND	ND	Pelengaris et al., 1999
Ins-MycER^T; RIP-Bcl-x_L	Tamoxifen	Pancreatic β cell carcinoma	6 weeks	Ubiquitous	ND	Regression	Apoptosis, vascular degeneration	ND	ND	Pelengaris et al., 2002
EμSR-tTA; tetO-Myc	Tet-off	T cell lymphoma, myeloid leukemia	13–22 weeks	Stochastic	Clonal	Regression	Cell cycle arrest, differentiation, apoptosis	Some tumors	Myc-independent	Felsher and Bishop, 1999; Karlsson et al., 2003
EμSR-tTA; tetO-Myc	Tet-off	Osteogenic sarcoma	13–22 weeks	Stochastic	ND	Regression	Cell cycle arrest, differentiation	ND	ND	Jain et al., 2002
LAP-tTA; tetO-Myc	Tet-off	Hepato-cellular carcinoma, hepato-blastoma	12 weeks (mean)	Stochastic	ND	Regression	Differentiation, apoptosis, tumor dormancy	Few tumors	L- or N-Myc overexpression	Beer et al., 2004; Shachaf et al., 2004
MMTV-rtTA; tetO-Myc	Tet-on	Mammary carcinoma	22 weeks (mean)	Stochastic	Clonal	50% complete regression, 50% incomplete regression	ND	100% of incomplete regressions and 50% of complete regressions	Myc-dependent and Myc-independent	Boxer et al., 2004; D'Cruz et al., 2001

dormant cell clones. The stem cell-like features of these cells are reflected by their capacity to differentiate into both hepatocytes and biliary cells. Upon reactivation of Myc, tumorigenesis quickly resumes. While this model confirms the critical relevance of a particular oncogenic pathway (Myc in this example), the outcome is very different from what was seen in the osteogenic tumor model mentioned above. In this liver tumor model, continuous suppression of Myc is necessary and sufficient to permanently suppress the tumorigenic potential of the hepatocytes.

But real life is usually not that simple. There was always the awareness that the growth of most tumors is fostered by too many lesions to assume that suppressing a single oncogene will be sufficient to cause long-lasting tumor regression. Therefore, the models described above might be the exception rather than the rule. Both clinical practice and other mouse tumor models testify to this. Overexpression of SV40 T-Antigen in the mandibular gland of mice causes ductal hyperproliferation that regresses upon termination of T-Antigen expression. However, when cells were exposed to T-Ag for longer periods, tumor regression did occur less efficiently, and polyploid cells remained, indicating that long-term T-Ag expression triggers additional mutations that by themselves can provide a condition from which tumor progression can ensue (Ewald et al., 1996).

In the current issue of *Cancer Cell*, inducible overexpression of MYC has been used to question whether mammary tumorigenesis induced by MYC shows the addiction observed in the systems described above (Boxer et al., 2004). In this study, tetracycline-inducible MYC expression is targeted to the mammary gland by the mammary-specific expression of the reverse Tet transactivator (rtTA) from the MMTV promoter. Chronic induction of mammary gland-specific MYC expression through administration of the tetracycline analog doxycycline results in mammary tumorigenesis. Downregulation of MYC following removal of doxycyclin causes regression of a fraction of the tumors. While in some of the tumors that only temporarily regress upon doxycyclin withdrawal, MYC expression has become independent on induction, others resume growth without requiring high levels of MYC and therefore have become refractory to MYC inhibition, suggesting that alternative pathways are triggered to compensate for the lack of Myc expression. Another subgroup of the tumors do regress, but become Myc-independent upon repeated cycles of induction and downregulation of Myc. Interestingly, almost 70% of the tumors that initially do not regress carry activating point mutations in the Kras2 gene. Kras2 was shown previously to act as the preferred partner of Myc in mammary tumorigenesis in an inducible Myc mammary tumor model (D'Cruz et al., 2001). Likely, the Ras mutations are instrumental in preventing tumor regression upon MYC downregulation. In contrast, only 25% of the tumors that recur upon repeated Myc induction and downregulation carry Kras2 mutations, suggesting that in this group, other lesions are better suited to overcome the MYC addiction. The picture that emerges from this study is that mammary tumorigenesis has its own rules that differ from those of the other models. In the mammary tumor model, Kras2 mutations (or mutations in the pathway) are likely needed to tolerate Myc overexpression. One might speculate that the hyperplasia induced by Myc overexpression permits sufficient cell expansion to allow such complementing mutations to take place. One also wonders whether Myc acts here as an initiating mutation or rather as a proliferative stimulus that allows the occurrence of other mutations that are actually more critical for fostering the oncogenic potential of progenitor cells, e.g., cancer stem cells. In analogy with the distinct roles of tumor-initiating and -promoting agents in chemical carcinogenesis, Myc might fulfill here a role of promoter rather than initiator, meaning that Myc overexpression might only promote, in a reversible fashion, malignant transformation of cells

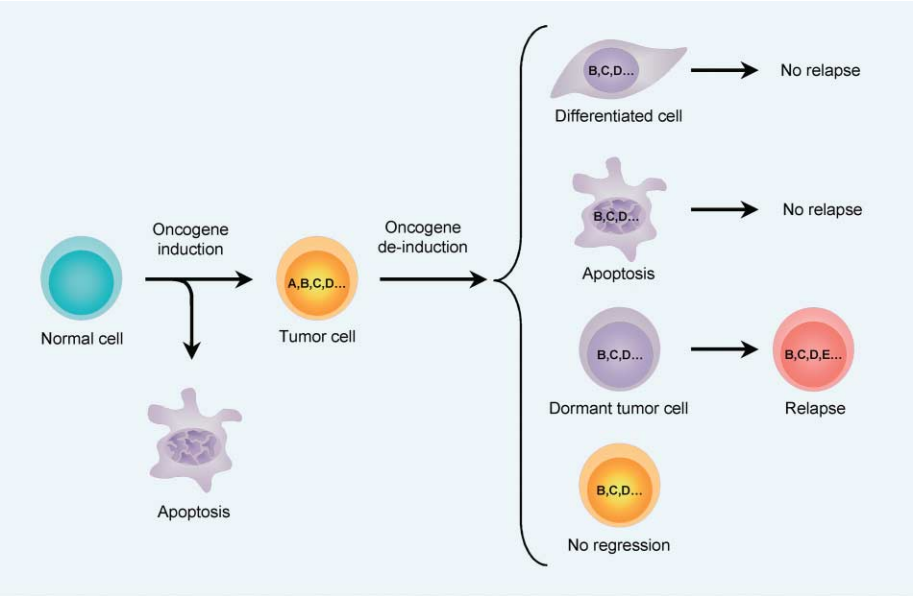

Figure 1. Different responses to oncogene inactivation

Tumors that develop in mouse models of oncogene inactivation show different responses to oncogene downregulation. Cancer cells can undergo differentiation or apoptosis, leading to sustained regression. Alternatively, cells can enter a quiescent state and resume growth upon acquiring additional mutations. Half of the MYC-induced mammary tumors are oncogene-independent and show no regression upon MYC inactivation. The capital letters A, B, C, etc. indicate the mutations acquired. A stands for the inducible oncogene that was deinduced in tumor-bearing animals to determine oncogene dependence of the ensuing tumors.

that have acquired initiating mutations. This would also be in line with the observation that in human mammary tumorigenesis, Myc amplification constitutes a tumor progression event rather than an initiating lesion (Robanus-Maandag et al., 2003). This then also raises the concern that the Kras2 mutations are artificially selected because of the widespread overexpression of Myc in otherwise normal mammary epithelial cells. Indeed, mutations in Ras are frequently seen in this model, whereas they are rare in mammary tumors in man.

What does this variation in responses to distinct oncogenic lesions teach us? First of all, that the concept of oncogene addiction, although intriguing, may have limited relevance for the majority of tumors. Secondly, that the nature of oncogene activation, i.e., massive overexpression to high levels in a large cell compartment, will cause cells to undergo different selection pressures than those occurring in spontaneous tumorigenesis. Nevertheless, although we should keep all these limitations in mind, the various models of oncogene inactivation point to underlying mechanisms that likely have general validity worth paying attention to.

The different consequences of brief or prolonged MYC inactivation in different cell types appear to correlate with intrinsic and/or acquired properties of the cells of origin (Figure 1). Tumor cells that have the capacity to undergo terminal differentiation and/or apoptosis following MYC downregulation may respond well to even brief MYC inactivation, as is the case for MYC-induced osteogenic sarcomas. This phenomenon of Myc might also relate to the capacity of Myc to distort the balance between stem cell self-renewal and differentiation leading to depletion of the stem cell compartment, as has been observed in the hematopoietic system (Wilson et al., 2004). On the other hand, cells that can persist in both the presence and absence of MYC overexpression might ultimately acquire mutations that lead to tumor recurrence, as is the case in MYC-induced hepatocellular and mammary carcinomas. The nature of the initiating oncogene appears to be of little influence on the response of the resulting tumors to oncogene inactivation, as relapses have been observed in animals with fully regressed mammary tumors induced by various Tet-regulatable oncogenes.

In both the liver model and the mammary tumor model, a portion of the cells survive Myc downregulation and show an enhanced capacity to reinitiate tumor growth, most likely due to distinct genetic or epigenetic changes. Identifying these dormant cell populations and the mutations therein is key, as they might embody the driving force for tumor relapse: a cancer stem cell compartment refractory to many of the drugs that have been designed to attack pathways critical for expansive growth, but that cannot eliminate dormant, nondividing cells that have acquired enhanced survival capacity. The question then is how we can target these dormant cells and elicit their destruction or differentiation. We will need to know more about the lesions critical to maintain this dormant state. Their long-term survival is remarkable, and ultimately, we need the tools to make these cells vulnerable to small molecule intervention. Therefore, it will be necessary to reconstitute this condition, preferentially in vitro, in which we now can genetically manipulate these dormant cells and screen for genes that show synthetic lethality with the mutations conferring survival of these cells.

Another lesson has to be learned from these studies. The fact that an oncogene can induce tumors does not qualify the oncogene as an initiating mutation, even though it is instrumental in the onset of the particular tumor. The confusion might arise because massive overexpression of oncogenes (in this case MYC) in a large number of cells that respond by proliferation creates a condition that will facilitate the occurrence of additional mutations. Those mutations that collaborate efficiently with MYC in fostering tumor growth will be preferentially selected. While such mutations might not allow survival of large tumor cell masses upon deinduction of MYC, they might confer a selective advantage on a stem cell compartment that has different requirements for cell survival. Kras2 might not be the most effective gene to confer this advantage. That would explain why the frequency of Kras2 mutations decreases in tumors that recur after repeated induction and deinduction of MYC. These still-to-be-found mutations might be specifically relevant for the maintenance of a tumor stem cell compartment.

These studies also emphasize a more general point relevant for modeling cancer in mice. Even though important principles can be established by models as discussed here, it will be important to test the validity of the conclusions in tumor models that more closely recapitulate the ontogeny of human tumors. This will require activation of oncogenes or inactivation of tumor suppressor genes, known to occur in early phases of human

tumor growth, in a sporadic fashion in the mouse model (Jonkers and Berns, 2002). To achieve both sporadic and inducible oncogene expression, one could employ retroviral delivery of the (reverse) Tet transactivator using the RCAS-TVA system (Pao et al., 2003). Alternatively, "latent" Tet transactivator alleles that become expressed only upon somatic recombination in vivo or Cre-inducible conditional versions may be employed. Once spontaneously occurring collaborating mutations found in the model are similar to those found in the cognate human tumors, one might expect that the model can predict some of the unique responses discussed here. These studies also emphasize the need to base cancer treatments on knowledge of the oncogenic lesions present in the tumor. In one case, a short treatment resulting in permanent differentiation or apoptosis of the tumor cells might suffice. In another case, it might be necessary to chronically suppress a critical oncogene on which tumorigenesis fully depends. In yet another setting, a combination treatment might be needed that specifically elicits a proliferative response of a tumor-prone stem cell compartment that is subsequently killed according to synthetic lethal principles.

Selected reading

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