Rolling the Dice to Discover the Role of DICER in Tumorigenesis

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In an effort to identify the consequences of complete DICER1 loss in tumorigenesis, in this issue of *Cancer Cell*, Ravi et al. characterize the effects of homozygous deletion of *Dicer1*. Using an in vivo mouse model, they show that genetic deletion of *Dicer1* does not preclude tumor formation.

MicroRNAs (miRNAs) are small noncoding RNAs that inhibit gene expression at the posttranscriptional level. miRNAs are synthesized in the nucleus by RNA polymerase II as long primary transcripts, termed primary miRNAs (pri-miRNAs). They are subsequently cleaved by DROSHA to release hairpin-shaped precursors of 70–90 nucleotides (nt) in length (pre-miRNAs). These are transported by Ran-GTP/EXPORTIN-5 (XPO5) to the cytoplasm, where DICER1 processes them to yield a 19–22 nt-long duplex. One strand of the duplex is loaded into the RNA-induced silencing complex (RISC), which delivers mature miRNAs to their mRNA targets. TARBP2, an RNA-binding protein that forms a complex with DICER, acts as a biosensor selecting the miRNA to be loaded into the RISC complex. It has been predicted that miRNAs regulate the translation rate of about half of all protein-coding genes, so their role in most biological processes, including development, metabolism, cell proliferation, differentiation, and apoptosis, is now well-recognized. For this reason, it is not surprising that deregulation of miRNA levels has been associated with tumorigenesis.

Although both tumor suppressor and oncogenic miRNAs have been described, the global downregulation of miRNAs is emerging as a common hallmark of cancer. Recent studies have highlighted possible mechanisms, including epigenetic and genetic events, which could explain this decrease in miRNAs in tumors. In the same way as previously described for protein-coding genes, several studies have identified CpG island promoter hypermethylation-associated epigenetic silencing as a common mechanism for miRNA repression in cancer (Saito et al., 2006; Lujambio et al., 2007; Davalos et al., 2012).

On the other hand, tumor-specific genetic defects in genes encoding members of the miRNA-processing machinery, such as TARBP2, XPO5, and DICER1, have been described. Truncating mutations in TARBP2, associated with destabilization of the DICER1 protein and consequent impairment of miRNA processing, have been identified in sporadic and hereditary carcinomas with microsatellite instability (MSI) (Melo et al., 2009). Also, in a subset of human tumors with MSI, XPO5-inactivating mutations that cause trapping of pre-miRNAs in the nucleus and impair the production of mature miRNAs have been detected in cancer cells (Melo et al., 2010). In the case of DICER1, heterozygous germline truncating mutations have been identified in families with the pleuropulmonary blastoma-inherited cancer syndrome (Hill et al., 2009) and hypomorphic somatic missense mutations have been detected in non-epithelial ovarian tumors (Heravi-Moussavi et al., 2012). However, the complete loss of DICER1 has not been reported. This fact and evidence from mouse models have led to the suggestion that total DICER1 depletion could be deleterious to tumor development.

In an effort to identify the consequences of complete DICER1 loss in tumorigenesis, Ravi et al. (2012 [in this issue of *Cancer Cell*]) characterize the effects of homozygous deletion of *Dicer1* using two cellular models: *Dicer1* null cells derived from a mouse sarcoma and those established from murine mesenchymal stem cells (MSCs) (Figure 1). To ensure accurate assessment, while preventing competition from cells that retain *Dicer1*, clonal isolation of $Dicer1^{-/-}$ cells was compulsory in both cases, since preferential outgrowth of heterozygous DICER1-expressing cells was detected after multiple passages. In comparison with parental heterozygous $Dicer^{f/-}$ cells, monoclonal homozygous $Dicer1^{-/-}$ cells derived from both models exhibited a global loss of mature miRNAs, a concomitant accumulation of precursors, derepression of miRNA luciferase reporters, proliferative lag, delayed exit from G1 phase of the cell cycle, and higher levels of basal apoptosis.

Having confirmed the tolerance of *Dicer1* depletion in vitro, to address the main issue at hand—the role of DICER1 in tumorigenesis—Ravi and coworkers developed an in vivo mouse model. $Dicer1^{-/-}$ sarcoma cells were injected into both immune-compromised and immunocompetent mice. In view of the growth disadvantage of *Dicer1* null cells relative to those retaining *Dicer1* expression detected in vitro, the authors accurately confirmed that the tumors were composed predominantly of $Dicer1^{-/-}$ cells. Strikingly, the experiments revealed that genetic ablation of DICER1 impaired but did not preclude tumor formation. $Dicer1^{-/-}$ sarcoma cells did retain their in vivo tumorigenicity, albeit at lower rates than $Dicer1^{f/-}$.

Considering previous publications and the apparently contrasting results obtained from other mouse models, one of the main findings of this study is the detection of the selective growth advantage of *Dicer1*-expressing cells over

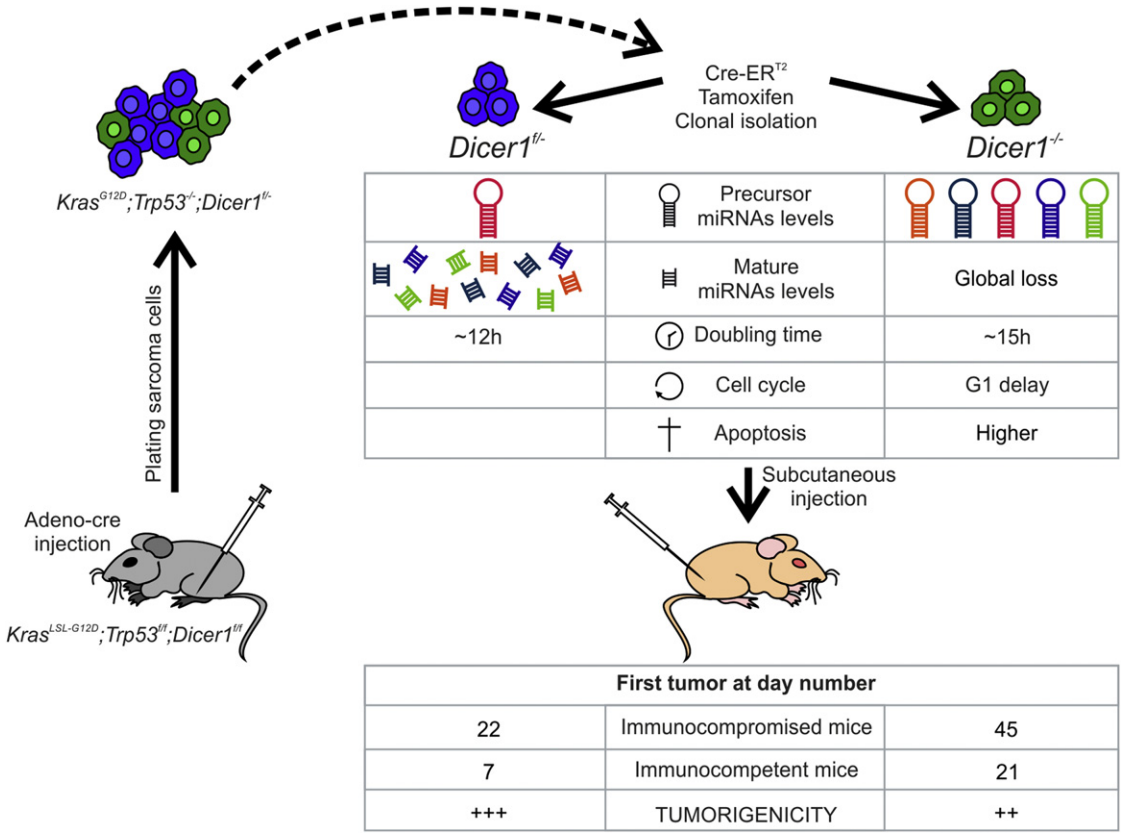

Figure 1. Characterization of the Effects of Homozygous Deletion of *Dicer1* Using a Murine Sarcoma Model

Dicer1-heterozygous sarcoma tumors were generated after hindlimb injection of Adeno-cre virus in Kras$^{LSL-G12D/+}$;Trp53$^{f/f}$;Dicer1$^{f/f}$ mice. Sarcoma cell lines were established and clones were isolated following Cre-ER integration and tamoxifen treatment. In comparison with parental heterozygous *Dicer*$^{f/-}$ cells, monoclonal homozygous *Dicer1*$^{-/-}$ cells exhibited a global loss of mature miRNAs with concomitant accumulation of precursors, proliferative lag, delayed exit from G1 phase of the cell cycle, and higher levels of basal apoptosis. Next, *Dicer*$^{f/-}$ or *Dicer1*$^{-/-}$ sarcoma cells were injected in the flanks of immunocompromised and immunocompetent mice. Although at slower rates relative to *Dicer1*$^{f/-}$, *Dicer1*$^{-/-}$ sarcoma cells retained their tumorigenicity in vivo.

Dicer1 null cells. A significant advance in our knowledge about DICER1's role in cancer has been achieved through the use of clonally isolated *Dicer1*$^{-/-}$ cells in the experiments described in this study. Thus, the interpretations of previous results, including those involving analyses of tumor samples, should be reconsidered since it is possible that subpopulations exist with different proliferative rates and because of the preferential outgrowth of cells expressing *Dicer1*. Ravi et al. (2012) present convincing evidence that *Dicer1*$^{-/-}$ cells are able to survive, proliferate without recovery of miRNA processing, and form tumors in mice. However, the occurrence of homozygous mutations in nature remains to be demonstrated, so the conclusions drawn from *Dicer1*$^{-/-}$ models must be considered cautiously. In addition, the experiments of Ravi et al. (2012) are limited to mesenchymal settings (sarcoma and MSCs), so their confirmation in other cell models is imperative, especially given the epithelial origin of most human tumors.

Certainly, however, the experimental models developed by Ravi et al. (2012) are a valuable tool for expanding our knowledge about the function of the miRNA processing pathway in the context of cancer. Consistent with previous findings from other genes of the miRNA pathway that show cancer-related loss-of-function mutations (Melo et al., 2009, 2010), as well as the well-recognized global downregulation of miRNAs in tumors, this study firmly supports the tumor suppressor role of the miRNA machinery. In this scenario, restoration of normal miRNA levels represents an attractive approach in cancer therapy. A new "miRNAome-based" strategy has been suggested, involving the use of the small molecule enoxacin. Proof-of-principle studies in human cancer cell lines and xenografted primary tumors have shown the powerful cancer-specific growth-inhibitory effect of this drug, mediated by the TARBP2-dependent restoration of the expression of tumor suppressor miRNAs (Melo et al., 2011). The global activation of miRNA processing by DICER1 stabilization with enoxacin has also been confirmed in neurons (Huang et al., 2012). The tumor suppressor role of miRNAs themselves and genes encoding members of the miRNA processing machinery warrants intensive research addressing the pharmacological approaches required to restore the global miRNAome in cancer.

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A Big Step for SIRT7, One Giant Leap for Sirtuins… in Cancer

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Recently reporting in *Nature*, Barber et al. demonstrated that SIRT7 maintains critical features that define cancer cells by removing the acetylation mark on lysine 18 of histone H3. Interestingly, hypoacetylation of H3K18 has been described as a general marker of tumor prognosis and oncoviral transformation.

Sirtuins are NAD^+-dependent deacetylases that target histone and non-histone proteins and are major factors in the response to oxidative, metabolic, and genotoxic stresses. Their responses are global and occur at many different levels; consequently, Sirtuins are at the crossroads among the foremost pathways that control cellular fate, including those for survival, genomic stability, apoptosis, and energy or metabolic adaptation. The importance of Sirtuins is reflected by their implication in several major human pathologies, including cancer, diabetes, cardiovascular diseases and neurodegenerative diseases (Bosch-Presegué and Vaquero, 2011).

Mammals have seven Sirtuins (denoted SIRT1–7) that have considerably different functions and catalytic activities. SIRT7 has been one of the most puzzling Sirtuins. Although researchers had clearly identified SIRT7 in chromatin, they had not found any clear catalytic activity or target specificity for it. The only target that had been proposed for SIRT7 was p53, but this is currently under debate. Evidence has supported a crucial role for SIRT7 in oxidative and genotoxic stress response. Homozygous knockout of *Sirt7* in mice causes diminished lifespan and leads to heart hypertrophy and inflammatory cardiopathy. Cardiomyocytes derived from these mice show increased apoptosis as well as hypersensitivity to oxidative and genotoxic stress. However, other than an ill-defined functional relationship between SIRT7 and p53 activity, no clear molecular explanation has been determined for these phenomena (Vakhrusheva et al., 2008b).

Another reported role for SIRT7 is in the control of ribosomal RNA (rRNA) expression. SIRT7 localizes mainly in the nucleolus, where it binds to the rRNA genes (rDNA) and participates in activation of RNA-polymerase I (pol-I) transcription (Figure 1A). Although this function apparently depends on SIRT7 having an intact "catalytic domain" (defined by homology to other Sirtuins), no mechanism has been described (Ford et al., 2006). However, some evidence suggests that this SIRT7 function may be specific to certain cell types (Vakhrusheva et al., 2008b; Barber et al., 2012). Interestingly, SIRT7 is relevant for the reactivation of rDNA transcription at the end of mitosis. Although the exact mechanism of SIRT7 action here is unknown, its interactions with the pol-I cofactors UBF and chromatin remodeling complex B-WICH have been described (Grob et al., 2009). Based on these findings and that SIRT7 is more abundant in highly proliferative tissues than in lowly proliferative tissues, a role for SIRT7 as a principal activator of proliferation has been proposed. On the contrary, other findings have suggested that SIRT7 may inhibit proliferation (Ford et al., 2006; Vakhrusheva et al., 2008a). This discrepancy has been a subject of controversy until now.

A recent report in *Nature* by Barber et al. (2012) represents a major breakthrough in SIRT7 research and redefines our view on the role of Sirtuins in cancer. The authors discovered a specific target of SIRT7 and identified a crucial role for SIRT7 in the maintenance of cancer phenotype and transformation. They found that SIRT7 is specific for a single histone mark, acetylated lysine 18 in histone H3 (H3K18Ac), directly linked to