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All You Need Is a Mir-acle: The Role of Nontranslated RNAs in the Suppression of B Cell Chronic Lymphocytic Leukemia

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miR-15a and miR-16-1 were the first microRNAs linked to cancer because their genes are commonly deleted in human chronic lymphocytic leukemia (CLL). In this issue of *Cancer Cell*, Klein and coworkers show that deleting a region with these genes in mouse provides a faithful model for human CLL.

Chronic lymphocytic leukemia is the most frequent leukemia of adults in the Western world. Loss of 13q14.3 distal to the retinoblastoma locus is the most common chromosome aberration in CLL, which is presented in the majority of cases (Döhner et al., 2000). Studies of clonal evolution in CLL indicated that heterozygous deletion of 13q14 is an early event, whereas deletion of the second copy of this region occurs at a later time point at a lower frequency (Stilgenbauer et al., 2007). Mutation analysis of protein-coding genes in this region revealed no inactivation of candidate genes. However, a complex epigenetic regulatory tumor-suppressor mechanism that would control the expression of the entire region and would account also for cases without 13q14 deletion has been proposed (Mertens et al., 2006). Deletions at 13q14 also occur at high frequencies in other lymphomas and solid tumors, such as mantle cell lymphoma, multiple myeloma, and carcinoma of the prostate and the lung, suggesting a major tumor-suppressor mechanism mediated by this chromosome region.

Calin and coworkers were the first to show that 13q14 deletion in CLL is associated with downregulation of miR-15a and miR-16-1, whose genes cluster in the minimally deleted region (MDR) within 13q14 (Calin et al., 2002). This was the very first link between miRNAs and cancer. Because each miRNA is expected to regulate the expression of hundreds of different genes, several studies have been carried out to identify the targets of miR-15a and miR-16-1 (e.g., Calin et al., 2008). Because the current algorithms for predicting targets via sequence similarities are imperfect and the effects of miRNA level changes measured in vitro are highly dependent on cell systems used, the physiological relevance of some of the published targets remains controversial.

Klein and coworkers now report on the conclusive functional test of relevant 13q14 genes in mouse models (Klein et al., 2010). The MDR in 13q14 contains the noncoding RNA gene *DLEU2* with the miR-15a and miR-16-1 cluster in its intron 4. Klein and coworkers generated sophisticated mouse models that have either deletion of DLEU2 together with both miRNA genes (MDR deleted) or deletion of the two miRNA genes only. After 15 to 18 months, about 5% of the animals displayed monoclonal B cell lymphocytosis (MBL), which is a possible precursor to CLL. More importantly, 1/5 of the MDR-deleted and 1/8 of the miR-15a/16-1-deleted mice developed CLL or the related small cell lymphocytic leukemia. In addition, 9% of the MDR-deleted and 2% of the miR-15a/16-1-deleted animals developed a phenotype reminiscent of human diffuse large B cell lymphoma (DLBCL), a disease known to progress from CLL at low frequency. Thus, the deletion of the MDR caused B cell lymphoproliferative disorders, nicely recapitulating the spectrum of human CLL phenotypes.

Notably, the MDR-deleted mice died significantly earlier than did their wild-type littermates whereas miR-15a/16-1 deletion alone did not result in survival differences. Thus, although both MDR-deleted and miR-15/16-deleted mice develop an indolent disease reminiscent of CLL, there is at least one genetic element within the MDR other than miR-15a/16-1 that modulates the aggressiveness of the disease. DLEU2 and the first exon of DLEU1 are the only known

transcribed sequences in the MDR interval besides miR-15a/16-1, and both genes generate transcripts that are not translated. Thus, the tumor-suppressor mechanism mediated within 13q14 clearly involves miRNAs as well as other noncoding RNAs.

The borders of the MDR were determined by comparing many CLL cases with 13q14 deletion. However, the vast majority of CLLs display much larger deletions. Interestingly, CLLs having the 13q14 deletion as the sole genetic abnormality have a favorable course of disease (Döhner et al., 2000). This is in sharp contrast to the poor prognosis of multiple myeloma, in which the same chromosome region is often deleted but is through a loss of the entire chromosome 13 in most of those cases. Thus, because sequences other than the miR-15a/16-1 cluster within the MDR are responsible for aggressiveness of CLL, sequence elements distant from the MDR on chromosome 13 seem to also seriously affect the clinical behavior.

Klein et al. also performed in vitro analysis of miR-15a/16-1 and DLEU2 by re-expressing them in a CLL cell line. Expression of DLEU2 had no effect on cell proliferation whereas expression of miR-15a/16-1 remarkably reduced cell proliferation and decreased expression of multiple genes involved in G0/G1-S phase transition, many of which are predicted to be targets of miR-15a/16-1. Failure of apoptosis induction is another important process in the pathophysiology of CLL (Korz et al., 2002). However, the in vitro studies by Klein et al. did not provide evidence for a role of these miRNAs in regulating antiapoptotic players such as BCL2 or NF-κB. Thus, deletion of miR-15a/16-1 seems to accelerate proliferation of B cells by regulating genes that control progression through the cell cycle. Similarly, a study on the role of miR-15a/16-1 in the pathogenesis of prostate cancer revealed *CCND1* and *WNT3A* transcripts as their targets and deletion of these miRNA genes promoting survival, proliferation, and invasion (Bonci et al., 2008). Furthermore, analysis of miR-15a/16-1 deletion in non-small cell lung cancer revealed a dominant role in cell cycle progression (Bandi et al., 2009). In this context it should be mentioned that, interestingly, the mouse models reported by Klein et al., which were deficient of MDR or miR-15a/16-1 in every cell, did not so far reveal evidence for neoplasms other than B cell lymphoproliferative diseases.

The pathophysiology of CLL shows a number of features that would make it very difficult to reproduce in a mouse model. (1) CLL is primarily a disease of elderly people. (2) It is an indolent lymphoma with high clinical heterogeneity, with many patients surviving for many years without serious symptoms. (3) It seems to be divided into two disease subclasses with pre- and postgerminal center B cell lymphocytes affected. (4) It has been suggested that a common antigen is involved in the etiology of CLL. All these features seem to be represented in the mouse models of Klein and coworkers. With the occurrence of the potential CLL precursor MBL, of CLL and small cell lymphocytic leukemia, and of a phenotype equivalent to DLBCL known to occasionally progress from CLL, the full variety of CLL-related diseases is present in these models. Furthermore, the disease occurs at advanced age with an indolent course, with the deletion of miRNAs alone not affecting survival of the organism. Based on the mutation analysis of immunoglobulin genes, it is evident that pre- as well as postgerminal center lymphocytes can be affected. Another highly interesting finding of the study is that detailed analysis of the immunoglobulin heavy chain revealed that both MDR-deleted and miR-15a/16-1-deleted mice can express antibodies with stereotypical antigen binding regions, a feature that is known for human CLL and is believed to indicate the existence of common CLL-inducing antigens or autoantigens.

Thus, these mice carrying deletion of the relevant chromosomal region in 13q14 mimic the biology of CLL in a highly intriguing manner, making them faithful animal models for CLL and superior to previously reported models. They also provide a paradigm for tumor-suppression mechanisms by noncoding RNAs, including miRNAs. Detailed characterization of these animal models will improve our understanding of the pathophysiology of CLL and related diseases. In addition, they might also become useful tools for testing novel therapy regimens.

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