MicroRNA Biogenesis Takes Another Single Hit from Microsatellite Instability

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In this issue of *Cancer Cell*, Melo et al. show that mutation of a single allele of the Exportin-5 gene (*XPO5*) suffices to depress microRNA (miRNA) levels and promote tumorigenesis. Thus, XPO5 joins the ranks of DICER and TRBP as a haploinsufficient tumor suppressor in the miRNA biogenesis pathway.

MicroRNAs are regulatory RNAs that silence mRNAs in a sequence-specific manner. Originally discovered in *Caenorhabditis elegans*, almost a thousand different human miRNAs are now known to repress a vast array of target mRNAs. As these include both oncogenes and tumor suppressors, dysregulation of miRNAs contributes to various cancers (Chang and Mendell, 2007). However, the causes of miRNA dysregulation are frequently unclear, particularly in those tumors where the levels of many or all miRNAs are changed. Possibly, such alterations reflect defects in the general miRNA biogenesis pathway.

MicroRNA biogenesis begins with the transcription of long primary miRNAs (Figure 1) (Kim et al., 2009). These pri-miRNAs are processed by the ribonuclease DROSHA (aka RNASEN) and its cofactor DGCR8/Pasha into the hairpin-shaped precursor miRNAs. DICER, in a complex with its partners TRBP or PACT, further processes the pre-miRNAs into short RNA duplexes. One of the duplex strands binds to an Argonaute protein to form an miRNA-induced silencing complex (miRISC), which represses target mRNAs through translational repression and degradation.

While pri-miRNA processing is a nuclear event, pre-miRNA processing occurs in the cytoplasm. Thus, pre-miRNAs need to transit from the nucleus into the cytoplasm, a process that requires the nuclear export receptor XPO5. Melo et al. (2010) now identify XPO5 as a haploinsufficient tumor suppressor in a subset of cancers with microsatellite instability (MSI^+). These cancers are characterized by expansion or contraction of short DNA repeats due to defects in the DNA mismatch repair system (Boland and Goel, 2010). In protein-coding sequences, frame-shift mutations result, which may render affected proteins nonfunctional and thus drive cancerogenesis through inactivation of tumor suppressor genes. MSI is particularly frequent in colorectal cancer (CRC), where it accounts for a combined total of 15% of sporadic and heritable cases, but also affects other sites.

Melo et al. (2010) report that *XPO5* is mutated in some MSI^+ cell lines and primary tumors. Expansion of an $(A)_7$ microsatellite in exon 32 alters and truncates the protein sequence and prevents XPO5 from both associating with its pre-miRNA cargo and exiting the nucleus. In $XPO5^{mut/+}$ heterozygous cells, less pre-miRNA was hence accessible to processing by DICER, resulting in decreased mature miRNA levels. The defects appeared to reflect loss, not neomorphic gain, of XPO5 function, since modest overexpression of wild-type XPO5 rescued the pre-miRNA export and processing defects. Interestingly, although the heterozygous XPO5 mutation decreased accumulation of a large fraction (~20%) of detectable miRNAs, many others remained unaffected. It thus appears possible that XPO5 does not bind to pre-miRNAs indiscriminately but has certain substrate preferences, perhaps mediated by sequence or structure. Downregulated miRNAs might then be those that bind poorly to XPO5 and thus cannot compete well for export by limiting amounts of XPO5. Alternatively, additional nuclear export pathways might be available to miRNAs that remained unaffected, as suggested by recent work in *C. elegans* (Büssing et al., 2010).

Several genes have been identified that are affected by MSI, but it is not always clear which of these are "driver mutations" that truly contribute to tumorigenesis and which are "passenger mutations" that signal the presence of a DNA repair defect but have little impact on tumorigenesis (Boland and Goel, 2010). Knudson's "two-hit" model offers a possible distinction (Payne and Kemp, 2005). It postulates that only inactivation of both alleles of a tumor suppressor genes (the two hits) provides cells with a selective advantage. Hence, true tumor suppressor genes will frequently display loss of heterozygosity (LOH), i.e., lose the remaining wild-type allele, in tumors. By contrast, lack of selective pressure on passenger mutations would typically cause these to remain heterozygous.

By this criterion, XPO5 would miss the bar. *XPO5* LOH could be observed neither in cell lines nor primary tumors. However, recent work has suggested that other components of the miRNA biogenesis pathway, DICER and TRBP (encoded by the *TARBP2* gene), are haploinsufficient tumor suppressors (Kumar et al., 2009; Melo et al., 2009; Hill et al., 2009). Moreover, biallelic deletion impaired cell viability, hence preventing LOH. This appears also true for XPO5: mimicking *XPO5* LOH by RNA interference (RNAi) against the *XPO5* wild-type allele in $XPO5^{mut/+}$ cells impaired cell viability. Conversely, supplementation of $XPO5^{mut/+}$ cells with wild-type *XPO5* from a transgene restored miRNA biogenesis to the wild-type situation and reduced tumorigenicity. Thus, it seems that at least three

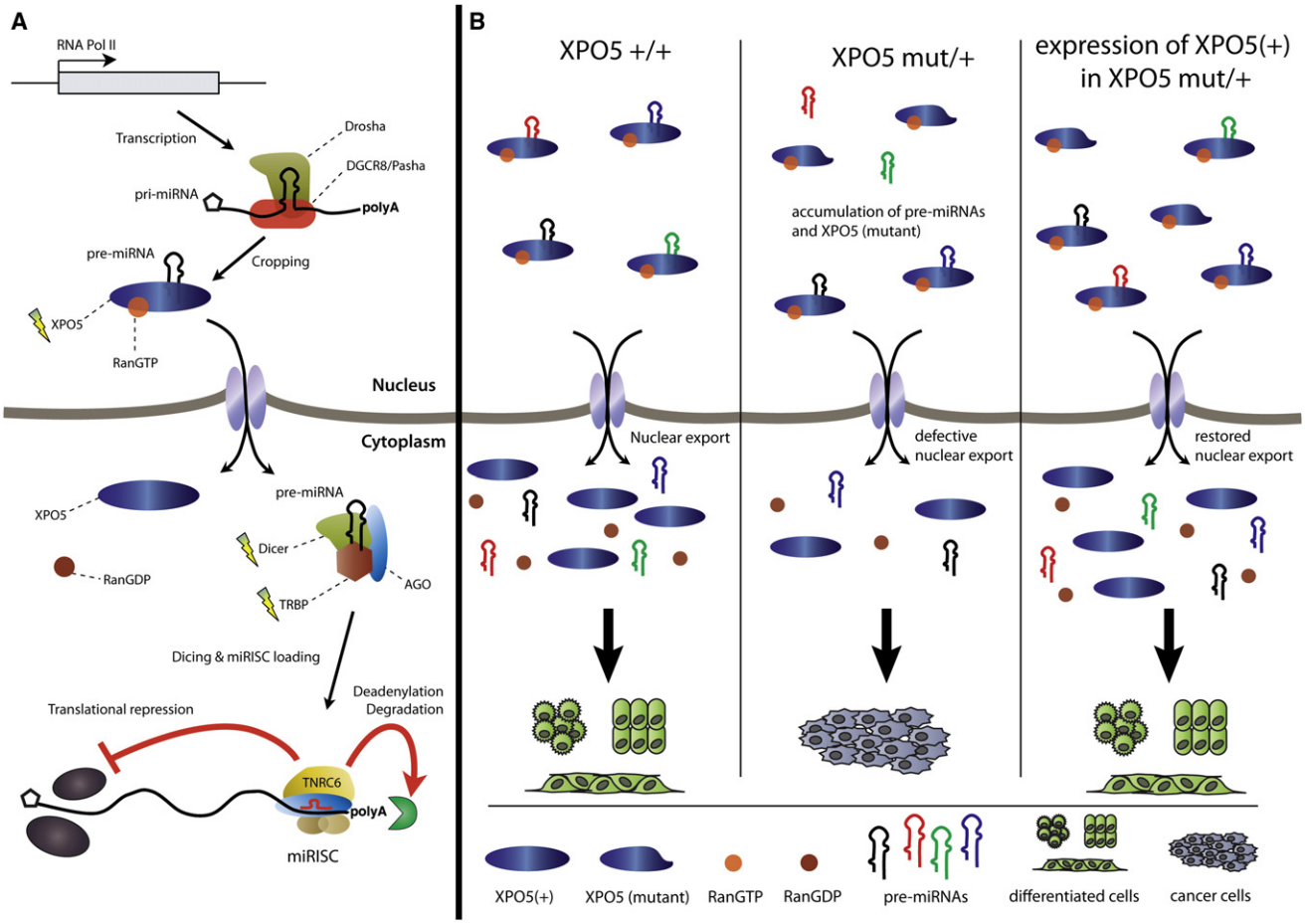

Figure 1. *XPO5* Haploinsufficiency Impairs miRNA Biogenesis and Promotes Tumorigenesis
(A) Schematic depiction of the miRNA biogenesis pathway. Some relevant proteins are indicated. XPO5 mediates nuclear export of pre-miRNAs in a complex with RanGTP; this complex disassembles in the cytoplasm upon hydrolysis of GTP to GDP. A bolt marks haploinsufficient tumor suppressors, which include *XPO5*. (B) Functional pre-miRNA export in $XPO5^{+/+}$ wild-type cells sustains the mature miRNA levels that are required to control cell differentiation and proliferation. Impaired pre-miRNA export in $XPO5^{mut/+}$ heterozygous mutant cells depletes mature miRNAs and is tumorigenic but can be overcome by expression of additional wild-type XPO5. This, and the fact that partial depletion of XPO5 by RNAi mimics $XPO5^{mut/+}$ heterozygosity, is consistent with haploinsufficiency rather than mutant gain of function causing the defects.

components of the miRNA biogenesis pathway are haploinsufficient tumor suppressors, with *XPO5* and *TARBP2* but not *DICER* mutations prevalent in MSI^+ tumors (Melo et al., 2009; 2010). In addition, the miRISC components *AGO2*, *TNRC6A*, and *TNRC6C* can be mutated in MSI^+ cancers (Kim et al., 2010), although functional consequences remain to be evaluated.

Is the case closed for *XPO5* and *TRBP* as haploinsufficient tumor suppressors in MSI^+ cancers? Not quite. So far, tumor suppressive function has been observed in vitro and in an allograft nude mouse model. In a next step, it will be important to demonstrate this function in $XPO5^{+/-}$ and $TARBP2^{+/-}$ hemizygous mice, respectively. Are these mice more prone to tumors, as observed for $DICER^{+/-}$ mice? If so, what kinds of tumors occur: gastrointestinal tumors as might be expected if the frequent *XPO5* and *TARBP2* mutations in MSI^+ cancers are consequences of specific selective pressures on these tumors? Would these mutations be more synergistic with CRC driver mutations than with mutations driving other tumors? Conversely, are *XPO5* and *TARBP2* subject to increased MSI rates in MSI^+ mouse models? If so, it may be possible to establish whether miRNA deficiency occurs at a specific point during tumorigenesis, perhaps driving one or more select events on the path to cancer.

Regardless of these possibilities, one important implication of the two studies by Melo et al. (2009, 2010) is that almost half of all MSI^+ tumors carry defects in their miRNA biogenesis machineries: among 337 primary tumor samples analyzed, some 23% carried one of three different microsatellite mutations in exon 32 of *XPO5*, all of which impaired XPO5 localization and miRNA biogenic activity, and some 26% of 282 primary tumor samples contained either one of two microsatellite mutations found to inactivate TRBP. Mutations in *TARBP2* and *XPO5* appear mutually exclusive. The presence of mutations in miRNA pathway genes in MSI^+ cancer samples, albeit at a lower frequency, has also been reported in a separate study of Korean patients (Kim et al., 2010). This begs the question of whether this pathway just makes a convenient target or whether a deeper

underlying rationale exists where miRNA depletion causes particular proliferative or survival advantages in these cancers. Indeed, it seems rather mysterious that a pathway that is considered important to provide robustness to gene expression programs would display itself so little robustness to mutational assault. It will therefore be of particular interest to examine whether haploinsufficiency is a general feature of miRNA pathway genes across various cancer sites and types or more closely restricted to MSI^+ tumors.

Finally, it remains to be seen whether the new findings can be exploited therapeutically. At this point, short of gene therapy, there seems to be little that can be done to target the *XPO5* defect directly so that restoring miRNA accumulation by alternative routes might be a more realistic approach. Provided that only one or few of the deregulated miRNAs are responsible for the tumor-promoting effect of *XPO5* mutation, it may be possible to supply them exogenously as miRNA duplexes that would not need to undergo nuclear export. Alternatively, it may be possible to identify a subset of key targets of the deregulated miRNAs that might be amenable to inactivation through classical pharmacological approaches or novel biologics.

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T-Lineage Lymphoblastic Lymphoma and Leukemia—a MASSive Problem

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T cell precursor malignancies may present as T-lymphoblastic lymphoma (T-LBL) with marked enlargement of lymph nodes or acute T-lymphoblastic leukemia (T-ALL) with little lymph node enlargement. In this issue of *Cancer Cell*, Feng et al. show that dysregulation of *BCL2*, AKT signaling, and cell adhesion pathways are hallmarks of T-LBL.

T-lineage lymphoblastic lymphoma (T-LBL) and acute lymphoblastic leukemia (T-ALL) represent up to 15%–25% of cases of ALL in children and adults and exhibit a remarkable spectrum of clinical, pathologic, and genetic features. In contrast to B-progenitor ALL, in which extensive bone marrow involvement at presentation is a near universal feature and significant lymph node enlargement is uncommon, patients with T-lineage disease frequently present with marked lymph node enlargement in the chest (mediastinal masses), often with minimal or absent bone marrow involvement (Figure 1). Traditionally, patients with a mediastinal mass and less than 25% leukemic cells (blasts) in the bone marrow are deemed to have T-LBL; those with a high marrow burden, T-ALL. While this distinction may appear somewhat arbitrary, a notable observation is that patients with large mediastinal masses frequently exhibit little, if any, evidence of tumor dissemination and marrow involvement, and the basis for this is unknown.

Both T-LBL and T-ALL cases commonly harbor chromosomal rearrangements, submicroscopic DNA copy number alterations, and sequence mutations. These alterations commonly dysregulate or disrupt genes with key roles in hematopoietic development, lymphoid differentiation, cell cycle regulation, and tumor suppression and are key events in leukemogenesis (e.g., rearrangements of T cell antigen receptor genes, *HOX11L1* and *HOX11L2*, *TAL1*, *LYL1,* mutation of *NOTCH1* and *FBXW7*, and deletion or mutation of *PTEN* and *WT1*) (Aifantis et al., 2008). However, in contrast to B-progenitor ALL, in which specific genetic alterations such as mutation of the lymphoid transcription factor *IKZF1* are strongly associated with poor prognosis (Mullighan et al., 2009), identification of features that predict clinical