From mRNA to tumor suppressor

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The combination of inhibition of RNA degradation and comparative genomic scanning is a powerful new method for detecting gene disruptions. The utility of the method is well-illustrated by a series of observations linking the ephrin receptor EPHB2 to prostate cancer.

The identification of tumor-suppressor genes has afforded great insight into the understanding of cancer and new methods of treatment¹. Cells have developed powerful ways to protect themselves from disruption of these genes. The recognition and repair of damaged DNA is one essential mechanism of protection. A second line of defense occurs at the RNA level. Nonsense-mediated decay (NMD) prevents synthesis of truncated proteins from transcripts that contain errors that disrupt open reading frames, including those that arise from mutations, from failed splicing or during normal transcription^{2,3} (Fig. 1). Stop codons that disrupt the reading frame are detected by the NMD mechanism and the mRNA is quickly degraded. NMD occurs predominantly in association with the nucleus and involves both failure of the first 'pioneer' ribosome to displace the final exon junction complex and enzymatic degradation. Protein synthesis inhibitors such as emetine, when applied for long periods, interfere with NMD and allow mRNAs with premature stop codons to accumulate. Noensie and Dietz⁴ showed that emetine could be combined with microarrays to screen for normally stable transcripts that are subject to NMD in a disease state, and therefore likely to be mutated. On page 979 of this issue, Pia Huusko and colleagues report the combination of emetine inhibition of NMD and microarray analysis with comparative genomic hybridization to screen prostate cancer-derived cell lines for transcripts that undergo NMD and are transcribed from genes with deletions on both alleles⁵.

Combine and conquer

EPHB2 is a receptor often associated with the establishment of tissue boundaries during development. Overexpression of EPHB2 is linked to a variety of cancers⁶, whereas loss of heterozygosity and mutations suggest that EPHB2 has a suppressor role in gastric carcinoma⁷. Using the new screening

method, Huusko *et al.* provide evidence that loss-of-function mutations in *EPHB2* occur in 8% of the 43 prostate cancers surveyed and that *EPHB2* has some of the hallmark properties of a tumor-suppressor gene.

The two-pronged approach is not always simple. Emetine has other pharmacological effects, as do all protein synthesis inhibitors, and these effects can complicate matters. Many, if not most, genes involved in disease have regulatory roles, and a disproportionate number of these produce transcripts that are short-lived, reflecting the usual case that the regulators themselves are tightly regulated. Most of these transcripts accumulate on inhibition of protein synthesis. Thus, drug-based repression of decay of any particular transcript in a disease must always be compared with similar repression in normal

cells, and induction of transcription must be taken into account⁸. NMD may also be used as a normal control mechanism, exerted through normal regulation of splicing into regions with disrupted reading frames⁹. This may complicate screening, in that disease states may sometimes show a shift from a stable mRNA isoform to another that is normally the target of NMD; the emetine strategy would presumably detect such a shift, which does not necessarily imply a new disease-related mutation. Whether this mechanism gets in the way depends on how often it is used and how globally the disease perturbs splicing. In any event, matters are sufficiently complex that sequence analysis will probably remain pivotal, and functional analysis will remain the ultimate arbiter. Finally, particular cell types

Figure 1 Newly spliced mRNA contains bound proteins of the exon junction complex (EJC), which are displaced by the ribosome in the pioneering round of translation. If a ribosome encounters a termination codon owing to mutation or splicing error while the mRNA still has a bound exon junction complex, this codon is recognized as a premature termination codon (PTC). This leads to the recruitment of several additional proteins that degrade the mRNA from both the 3' and 5' ends. The alkylloid emetine binds to ribosomes and blocks nascent peptide elongation, which halts ribosome progress and contact with premature termination codons, thereby stabilizing the RNA of mutated genes. DCP2, 5' decapping protein; PARN, polyadenylate ribonuclease; Pm/Scf100, 3' to 5' active subunit of the exosome that catalyzes 3' to 5' mRNA decay.

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with a preponderance of nuclear NMD may require optimized protocols that promote efficient accumulation of nuclear mRNA.

EPHB2 in cancer

Mutations in the gene encoding EPHB2 were detected in multiple samples of metastases from the same individual, but none were detected in primary tumors, leaving open the question of the relation of the biallelic alteration to the cause of the tumor. This is important because numerous genetic alterations occur during the progression of most solid tumor to the metastasis stage. Nevertheless, as pointed out by Huusko *et al.*, recurrent sequence mutations in the same gene observed in multiple clinical samples of a case

are rare, consistent with a common early antecedent. The incidence of mutations in *EPHB2* (8%) does not compare with that of p53, which seems to be inactivated in >50% of all solid tumors⁶, indicating that the clinical impact of the inactivation of EPHB2 may be more limited.

The discovery of a new tumor-suppressor gene and association of the gene with a disease is always cause for celebration, but the invention of a new approach, or combination of approaches, to identify many more disease-gene associations is cause for genuine excitement, despite the few caveats mentioned above. As many as one-third of inherited disorders are caused by mutations that disrupt reading frames. Loss of function in tumor

suppressors is a key factor in cancer. As Noensie and Dietz point out⁴, the emetine inhibition of NMD is probably most useful as adjunct data to other genome positional information, such as the comparative genomic hybridization approach used by Huusko *et al.*⁵.

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