Cell

Noncoding RNAs and Cancer

Knockout Punch for Cancer

Judy Lieberman
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A decade after RNAi was found in mammals, clinical studies using microRNA antagonists and lipid-encapsulated siRNAs are showing promise for inhibiting hepatitis C virus replication and knocking down genes in the liver. The biggest challenge to RNA-based cancer therapeutics remains delivery into cells outside of the liver, which is critical for treating disseminated cancer. Attractive approaches to targeted delivery being developed avoid liposomes and use RNAs covalently linked to small-molecule conjugates or RNA aptamers. siRNA cocktails can sidestep the dangers of cancer escape mutations, since multiple mutations generally compromise fitness. The choice of siRNA targets may need to be customized based on each tumor's unique dependencies; siRNA drugs are ideally suited to personalized therapy. siRNA cocktails could be selected, based on a tumor's molecular fingerprint, from a siRNA pharmacy of the future containing hundreds of RNAs. In addition, mimics of tumor suppressor microRNAs (which have the same delivery issues as siRNAs) could provide a way to manipulate large networks of genes to inhibit tumor proliferation or metastasis or induce differentiation. It will be difficult for tumors to escape from these broadly acting RNAs. It is becoming clear that long noncoding RNAs regulate transcription, maintain heterochromatin, promote enhancer function, and help to repair DNA damage—all processes central to malignant transformation and cancer treatment. RNA-based drugs that manipulate these newer noncoding RNA functions provide a therapeutic opportunity worth exploring.

The Promise of Small RNAs

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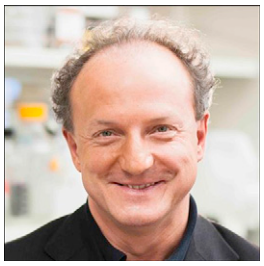
From the initial reports of microRNAs and RNAi in *C. elegans*, it is now clear that small RNAs play big regulatory roles in almost all species and regulated processes. Given their roles in regulating key human disease genes, they now show promise as therapeutics for a whole range of human ills. Our understanding of microRNAs in cancer is best developed, and even though the first human microRNA was only discovered in 2000, the field has already moved past the discovery stage into the translational phase. Because certain microRNAs are overexpressed in cancer and have oncogenic properties (oncomiRs), whereas others are lost in cancer and have tumor suppressor (TS) properties, the field has a two-pronged strategy: inhibit the oncomiRs (therapeutic target strategy) and restore the TS microRNAs (targeted therapeutic strategy). Both strategies show promise alone in mouse models of cancer, but it is likely that a combination of the two will be required for efficacy in the heterogeneous background of human cancer. This nascent field has benefited immensely from the prior knowledge and chemical innovations developed for oligo and siRNA therapeutics, and it suffers from some of the same drawbacks and challenges. Enhancing effective delivery and stability while eliminating potential side effects are perennial issues being addressed with novel nanoparticle strategies. The ingenuity of nanoengineering is highly likely to make this promise a reality for patients.

Genomic Dark Matter



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I believe that cancer research, diagnosis, and care will be revolutionized by the impact of the noncoding RNA dimension. However, despite intensive research, the functions of the noncoding portion of the genome have largely remained uncharted territory. We discovered that pseudogenes, noncoding RNA species, and, importantly, protein-coding mRNAs can function as competitive endogenous RNAs (ceRNAs) and compete for a limited pool of microRNAs. Such competition leads to crosstalk that can be predicted based on number and identity of shared microRNA response elements (MREs), the letters of the "MRE language," and allows the formation of complex regulatory networks. Such networks are crucial in lending robustness to evolutionarily conserved biological systems while permitting speciation and diversification but also promote disease when their balance is perturbed. The identification of the ceRNA dimension will allow us to unravel and functionalize large portions of the "dark matter" of the genome. Once such knowledge has been integrated with our model of the protein-coding world, we will be one step closer to understanding the biology of cells. We are currently approaching this by interrogating one noncoding RNA at a time in cells and animal models. I believe, however, that bringing together systems biologists, mouse modelers, bioinformaticians, and molecular biologists will more rapidly identify the critical nodes in these complex networks and their contribution to disease. This, in turn, will provide novel potential drug candidates that can be explored for therapeutic purposes.

lncRNAs Complicate Cancer

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Emerging evidence suggests that the vast, often uncharted, noncoding landscape of the human genome plays an important role in biology and disease progression. Small ncRNAs, such as microRNAs, have well-established differential patterns of expression in cancer and function as tumor suppressors or oncogenes by silencing target gene expression. Much less is known about their longer cousins, long noncoding RNAs (lncRNAs), subsets of which have been characterized as epigenetic factors, enhancers, antisense transcripts, and pseudogenes. The popularization of RNA-seq technology to interrogate the transcriptome has revealed the pervasive expression of lncRNAs in cancer progression. While the research community is still in the early days of characterizing lncRNAs across tumor types, lineage-specific and sometimes cancer-specific patterns of expression are emerging.

The function of most lncRNAs in cancer remains a mystery. Prototypical lncRNAs have been shown to be overexpressed in subsets of cancers, interact with epigenetic complexes, be recruited to DNA sequences as enhancers, and function in RNA-RNA hybrids. A major challenge in understanding the function of a specific lncRNA in cancer is deciphering its "interactome"—whether it be with specific protein complexes, regions of DNA, or other RNAs. Several high-throughput technologies are now emerging to address this gap. Ultimately, lncRNAs will likely have implications in cancer management, including the development of more specific cancer biomarkers, due to their lineage- and cancer-specific properties.

Assessing RNA Therapies

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The past decade has witnessed a swift advance in the identification of microRNAs with disease-specific expression patterns and those with causative roles in disease development and—in the case of cancer—in resistance to therapy. This has led to the initiation of clinical trials testing oligonucleotide-based therapies inhibiting or mimicking microRNAs. With their simple design, scalability, and systemic delivery to the body, oligonucleotide-based therapy is easier to develop, improve, and put to practice than gene- and drug-based therapies. The outcome of these trials will determine how valuable microRNAs are as therapeutic targets.

Concerning cancer, I thus believe that the greatest challenge ahead is to convincingly demonstrate efficient oligonucleotide delivery accompanied by long-lasting effects on tumor cells in patients. Particularly, specificity and safety of treatment should be demonstrated, as some microRNAs can function as both oncogenes and tumor suppressors, depending on the targeted tissue.

In contrast to microRNAs, the research field of lncRNA lags far behind. Their recent identification, long size, low expression level, and excessive structure delay progress. Although they are as powerful as microRNAs in controlling essential cellular processes, dedicated preclinical investigation of lncRNAs is required to decode the language that they talk and the words they use in order to discover the best ways to control their function. Only when such a pronounced fundamental understanding is achieved can we start implementing lncRNAs in therapy.

Revealing ncRNA Disease Roles

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Over the last decade, numerous studies have documented the potent pro- and anti-tumorigenic functions of noncoding RNAs (ncRNAs) and, particularly, microRNAs (miRNAs). Understanding how aberrant ncRNA activity mechanistically contributes to tumorigenesis and predicting the sequelae of targeting ncRNAs therapeutically require a deeper understanding of the roles of ncRNAs in normal physiology in vivo. Functional studies of miRNAs and other ncRNAs in knockout and transgenic mice have repeatedly revealed surprisingly subtle phenotypes that can be enhanced by various forms of stress, including acute injury and chronic disease. Based on this emerging paradigm, animals with ncRNA knockout or overexpression should be exposed to broad panels of perturbations to reveal defective pathways that might not be apparent in controlled laboratory environments. Mechanistically dissecting these phenotypes in whole animals poses a daunting challenge. It is often stated that miRNAs fine-tune the expression of many messenger RNAs simultaneously. Likewise, long ncRNAs are often components of broadly acting chromatin-modifying complexes. Subtle regulation of a large number of targets may, in aggregate, result in profound effects on cellular behavior. Yet it remains possible that many ncRNA-driven phenotypes result from the regulation of a few key targets hidden within these extensive networks. Uncovering these critical nodes, should they exist, will be essential for understanding ncRNA functions in physiology and pathophysiology. Moreover, these crucial effectors of ncRNA activity may themselves represent druggable targets, potentially offering greater specificity and ease of targeting than ncRNAs themselves.