Targeting siRNA to arrest fibrosis

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Vitamin A–mediated targeting of small interfering (si)RNA to stellate cells reverses liver cirrhosis.

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Hepatic fibrosis is a scarring response to injury that underlies the development of end-stage liver disease, or cirrhosis, in hundreds of millions of patients with chronic hepatitis worldwide. Despite substantial progress in uncovering the cellular and molecular mechanisms of fibrosis, to date there are no approved therapies. In this issue, Sato *et al.*[1] serve up a technical *tour de force* that could bring us closer to this elusive goal. Using several rodent models of hepatic fibrosis, they demonstrate a novel system for delivering an antifibrotic siRNA to hepatic stellate cells—the primary source of extracellular matrix, or collagen scar tissue, produced in response to liver damage[2] (**Fig. 1**). A dramatic reversal of fibrosis and cirrhosis was observed after several treatments, attesting not only to the therapeutic potential of this approach but also to the remarkable resilience and regenerative capacity of liver.

This study is among the first to successfully target stellate cells *in vivo*, using an elegant liposomal delivery system that exploits the uptake by these cells of vitamin A linked to liposomes. The liposomal targeting complex cleverly incorporates two critical constituents: vitamin A and an siRNA against the collagen chaperone heat shock protein 47 (HSP47).

Inclusion of vitamin A confers a high degree of specificity for hepatic stellate cells, the primary site for storage of dietary vitamin A (retinoid). Ordinarily, dietary retinoids delivered to the liver are first processed by hepatocytes, the major parenchymal cell type, and then transferred to stellate cells via a retinol-binding protein–dependent pathway[3]. Thus, it is interesting that the vitamin A–coupled liposomes were not first recognized by hepatocytes but instead delivered intact directly to stellate cells bound to retinol-binding protein. This was confirmed using gel filtration and by the capacity of antibodies to retinol-binding protein to inhibit uptake of the liposomes. Although some uptake by hepatic macrophages was noted, the absence of target sequence for the siRNA minimizes the impact of nonspecific uptake in these phagocytic cells. No uptake was seen in retinal cells, which are also rich in retinoids, presumably because the blood-brain barrier prevents the liposome complexes from reaching the eye.

The choice of HSP47 as the siRNA target is intriguing, as only a few studies have examined its role in liver fibrosis. HSP47 expression in the endoplasmic reticulum correlates closely with collagen production, and in liver is localized to collagen-producing activated stellate cells[4–6].

Having first established the rationale for inhibiting collagen production by knocking down this chaperone in cultured cells, the authors extended their investigations to three mechanistically distinct *in vivo* models. These involved using bile-duct obstruction or administering dimethylnitrosamine or carbon tetrachloride, each of which mimics cirrhosis. An impressive number of control experiments were performed to confirm the specificity of gene targeting and to exclude off-target effects due to an endogenous interferon response.

The effects of the liposome complex on liver fibrosis were striking and required all components—liposome, vitamin A and siRNA—for activity. Using three models to ensure that the effect of HSP47 antagonism was relevant to all etiologies of liver fibrosis, the authors found that repeated intravenous administration of the complex dramatically reversed advanced fibrosis, eliminated features of severe liver disease and prolonged survival. Although reduced collagen secretion was a major consequence of the treatment, there was also an increase in collagenase activity, which was essential to degrade the large mass of scar that had already accumulated.

The molecular basis for the antifibrotic effect of increasing matrix degradation is obscure and among the most intriguing findings of the study. Although efforts to combat hepatic fibrosis by targeting production of collagen, a major constituent of the hepatic scar, have been considered for decades, enthusiasm had waned more recently owing to the view that targeting a single participant in the complex cellular milieu of fibrosis would be insufficient to reverse the disease. The findings of Sato *et al.*[1] thus raise speculation whether HSP47 antagonism affects other targets besides collagen, such as elements of the immune system, or whether the downstream impact of inhibiting collagen deposition by this route is far broader than previously thought. One way to identify unanticipated targets of HSP47 knockdown would be to use microarrays to survey the molecular effects of the liposome complexes in cultured stellate cells. Perhaps reduced expression of collagen would alter the balance between matrix production

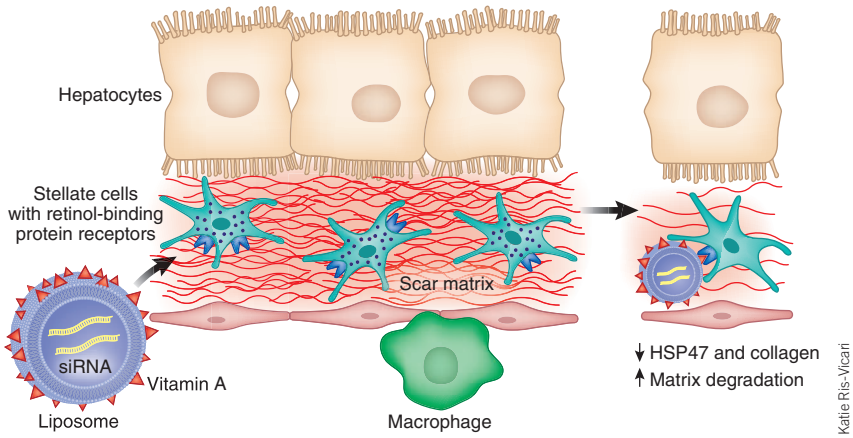

Figure 1 Delivery of antifibrotic vitamin A–coupled liposomes to hepatic stellate cells. siRNA directed against heat shock protein 47 (HSP47) is delivered through the circulation to activated hepatic stellate cells, where it binds to retinol-binding protein receptors, attenuates collagen production and promotes degradation of extracellular matrix, thereby reversing fibrosis and cirrhosis.

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and degradation by downregulating tissue inhibitors of metalloproteinases, which are a critical switch in mediating both matrix degradation and stellate cell survival[7].

Another implication of the study is the potential use of this targeting complex to administer other therapies directly to hepatic stellate cells, including other siRNAs or small molecules. The latter might be particularly useful to deliver small antifibrotic molecules, such as hepatocyte growth factor (HGF), while minimizing their impact on other cell populations, as untargeted HGF carries the theoretical risk of enhancing liver carcinogenesis through stimulation of hepatocyte mitogenesis. Moreover, as our appreciation of the range of functions of the hepatic stellate cell increases[1], indications other than fibrosis could emerge, such as enhancing regeneration or modulating immune responses. Finally, the complex could be used to deliver diagnostic or imaging molecules for assessment of fibrosis, currently a major unmet clinical need[8].

Although the results of this study are both technically and conceptually impressive, it seems unlikely that this targeting complex will become a widely used therapy for chronic hepatic fibrosis, as a range of experimental oral or parenteral therapies currently seem more appealing. Nonetheless, one could envision its use as an initial therapy to achieve rapid, potent antifibrotic activity followed by oral therapies to sustain the therapeutic benefit. Clearly, this technology opens new doors to improved understanding, diagnosis and treatment of hepatic diseases.

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Cancer biomarker profiling with microRNAs

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MicroRNAs define tissues of origin in cancer.

With the advent of high-throughput technologies for the global measurement of microRNAs (miRNAs), these post-transcriptional regulatory molecules are emerging as a new class of cancer biomarkers. Numerous studies have explored associations between miRNAs and cancer features. In this issue, Aharonov and colleagues[1] use miRNA expression levels in multiple primary and metastatic cancers to construct an miRNA classifier that identifies cancer metastases by their site of origin.

Discovered in *Caenorhabditis elegans* in 1993 and formally named in 2001 (ref. 2), miRNAs have been identified in every plant and animal species examined. They are a class of noncoding RNAs, 18–25 nucleotides in length, that plays key roles in the regulation of fundamental cellular processes such as differentiation, proliferation, apoptosis and metabolic homeostasis. The functions and targets of most miRNAs await discovery. However, specific miRNAs show expression variation across different stages of organism development, in tissue-specific cell patterning and asymmetry during organogenesis, and in oncogenesis[3].

miRNAs are encoded by DNA that may be situated in the exons or introns of genes or scattered among intergenic DNA. After their initial transcription in the nucleus they undergo a maturation process that involves (i) nuclear processing into a primary miRNA (pri-miRNA) and then a precursor (pre-miRNA); (ii) export into the cytoplasm; (iii) further processing into mature miRNA; and (iv) incorporation into an RNA-induced silencing complex (RISC) with an Argonaute protein catalyst (**Fig. 1**). The miRNA-RISC complex hybridizes to nucleotide sequences of varying complementarity in the 3′ untranslated region (UTR) of mRNA and inhibits protein synthesis or degrades the target mRNA[4]. In contrast to the ~20,000 genes in the human genome, 541 human miRNAs have been identified[5] with up to 1,000 predicted.

Why are miRNAs potentially important in cancer? First, in cancer their expression is dysregulated. Some miRNAs that are temporally overexpressed in early development and shut off in the normal differentiated state may become reexpressed in cancer, causing a persistent stem cell–like dedifferentiated state. miRNAs overexpressed in cancer may act like oncogenes by promoting proliferation and/or repressing apoptosis. Conversely, other miRNAs with tumor-suppressor regulatory functions in normal tissues may become downregulated in cancer, abrogating their tumor-suppressor activity.

Second, miRNA expression may provide unique insight into a tumor's origins. Although it is arguable whether miRNA expression is globally higher in normal tissues compared with cancers, both normal and malignant tissues have specific miRNA signatures and show differential expression across tumor types, with associations along tissue development lines (separate epithelial and hematopoietic dendrogram branches when samples are clustered, with further partitioning by cell-type lineage)[6,7]. Finally, overexpression or lack of expression of specific miRNAs appears to correlate with clinically aggressive or metastatic phenotypes[8,9].

Reasoning that miRNA expression has tissue specificity and has been used for tumor classification, Aharonov and colleagues[1] applied miRNAs to define tumor identity. They constructed an miRNA-based tissue classifier by measuring miRNA expression level using a microarray platform in 336 primary and metastatic tumors representing 22 different cancer types. Based on separate training and test sets of tumors and metastases, they built and tested a classifier of 48 miRNAs that accurately predicted tissue type in 86% of the test set, including 77% of the metastatic samples. Moreover, the classifier predicted tissue type with 100% accuracy for six of the ten tumor types in the metastatic test set. The authors proposed that their classification system could be applied to cancer of unknown primary origin, defined as histologically confirmed metastatic cancer for which no primary site of disease can be identified. Cancer of unknown primary origin accounts for ~2–6% of invasive malignancies and is a frustrating diagnosis for patients, families and treating physicians; prognosis is generally poor and treatment recommendations are unclear.

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