

Molecular Biosafety

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The molecular biology and biotechnology fields are growing by leaps and bounds. Molecular Biosafety aims to shed light on how these cutting-edge techniques impact safety. Please e-mail your insights and questions to Margy Lambert at mlambert@mcw.edu or Co-Editor Barbara Johnson at barbara_johnson@verizon.net or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

Safety Overview of Techniques Involving miRNAs, siRNAs, and Other Small Regulatory RNAs

Knowledge of miRNA function is rapidly expanding and new uses of miRNAs, siRNAs, and shRNAs as tools in biotechnology are emerging on a regular basis. MicroRNAs (miRNAs), small interfering RNAs (siRNAs), and short hairpin RNAs (shRNAs) are all small nonprotein-coding RNA molecules. While miRNAs are endogenous, siRNAs and shRNAs have been developed as tools to interact with mRNA (messenger RNA) to repress translation of mRNA into proteins. The miRNA field of study is relatively new with most work being done since 2000. As an evolving field, there are many unknowns about miRNAs including safety factors, but one clear take-away message is that miRNAs serve as important regulators of gene expression (Ying et al., 2008). Specifically, miRNAs have been proposed to regulate up to 90% of human genes through miRNA-guided RNA silencing (Perron & Provost, 2008).

miRNAs are transcribed in the nucleus as pri-miRNAs (primary microRNAs), then transported to the cytoplasm where the pri-miRNAs are processed to mature miRNAs which can then bind to mRNA targets to repress translation, alter mRNA cleavage, and promote mRNA decay/degradation. siRNAs and shRNAs are short RNAs manufactured to bind with mRNAs to prevent or interfere with translation. piRNAs (piwi-associated RNAs) are a class of small RNAs associated with germline development (Klattenhoff & Theurkauf, 2008).

The central dogma of molecular biology is that DNA is transcribed into mRNA which is then translated into proteins. In addition, DNA copies are made (replication) and RNAs other than mRNA (transfer RNAs and ribosomal RNAs) are involved in translation. Some proteins serve as regulators of multiple processes involving nu-

cleic acids such as transcription factors (regulators of gene expression). In reality, RNA molecules carry out a variety of functions in the cell. The delay in understanding miRNA function probably had a lot to do with the focus on the central dogma with the idea that the roles of endogenous RNAs were already understood. RNA playing a key role as a regulator of gene expression was a revolutionary idea.

miRNAs serve as functional counterparts of transcription factors—both are endogenous regulators of gene expression. While transcription factors regulate gene expression by interacting with DNA (up and down regulation of transcription), miRNAs regulate gene expression through interaction with mRNA (preventing translation, altering mRNA cleavage processes, and promoting mRNA degradation or decay). Use of siRNAs to inhibit translation pre-dated the understanding of the regulatory function of miRNAs. Studies targeting miRNA regulation have focused on RNA interference (down regulation of gene expression), but miRNAs and synthetic double-stranded RNAs (dsRNAs) can also act as RNA activators (up regulation of gene expression) (Pushparaj et al., 2008; Wu & Belasco, 2008).

miRNAs operate in a multitude of cellular and physiological processes including cell cycle regulation, embryogenesis, development of organ systems, differentiation, oncogenesis, tumor suppression, apoptosis, angiogenesis, toxicology, and immunity. Because of the wide-ranging effects of miRNA regulation, techniques to manipulate miRNAs are being successfully explored as treatment options for many diseases and disorders including cancer, infectious diseases, neurological disorders, metabolic disorders (e.g., diabetes), and cardiovascular and respiratory diseases.

Clinical oncology applications for miRNA technology are numerous since miRNAs can act as oncogenes and as tumor suppressor genes (Zhang et al., 2007) and play roles in cell cycle progression, apoptosis, angiogenesis, and metastasis (Fish & Srivastava, 2009; Gramantieri et al., 2008; Hermeking, 2007; Oulas et al., 2009; Tong et al., 2005).

Studies on the RNA-based immune system (small RNA-directed viral immunity) that is mechanistically related to RNA interference (Aliyari & Ding, 2009) have led to clinical applications for infectious disease prevention

and treatment (He et al., 2008; Park et al., 2005; Tang et al., 2008).

siRNA technology has identified some safety concerns that are relevant to miRNA studies; these include cellular toxicity, extended stability, insertional mutagenesis if the delivery system is a viral vector that integrates, effects in non-targeted organs, and off-target effects with altered expression of genes other than the targeted gene. Delivery systems are being developed to minimize the cellular toxicity that occurs primarily due to the triggering of innate immune responses (Akinc et al., 2008; Behlke, 2006; Boudreau et al., 2008; Judge & McLachlan, 2008).

RNAs are inherently unstable. Unmodified siRNA has a half-life of less than an hour in human plasma, and circulating siRNAs are rapidly excreted by the kidneys due to their small size (Layzer et al., 2004; Li et al., 2006). Delivery systems are being developed that extend the life of the regulatory RNAs to expand their utility in treatment (Frieden & Orum, 2006), but from the safety standpoint, longer-lived RNA could lead to unintended deleterious effects. In addition, if the delivery system chosen is a viral vector, the potential infectivity hazard of replication-competent virus generation exists with the additional potential hazard of insertional mutagenesis if the viral vector is one that integrates into the host genome (Li et al., 2006). On a separate but related topic, traditional assessment of insertional mutagenesis risk focuses on insertional effects on protein-coding genes. With the growing understanding of miRNAs' regulatory functions, assessment of insertional mutagenesis risk should also include the potential effects of vector insertion near non-coding sequences such as miRNAs.

Some *in vivo* studies with siRNA have demonstrated unwanted side effects due to the accumulation of siRNAs in organs other than the targeted one (Landen et al., 2005; Li et al., 2006). Several groups have successfully attempted to improve tissue-specific delivery of siRNAs by using either ligands for specific receptors or antibodies against cell surface markers (Li et al., 2006; Schifflers et al., 2004; Zhang et al., 2003).

Specific miRNAs, like specific transcription factors, can regulate the expression of a number of different genes, and because complex pathways of regulation are involved, altering the regulation of a miRNA, like altering the regulation of a transcription factor, can have multiple consequences, some of which are unintended. Regulatory molecules are often part of a complex cascade of events, individual miRNAs can regulate a number of different mRNAs with effects on different cellular processes, and an exhaustive list of which mRNAs are regulated by any specific miRNA is often not known (Marquez & McCaffrey, 2008). miRNA-like target interactions can be triggered by extremely limited sequence homology resulting in off-target effects (Jackson et al., 2003; Li et al., 2006; Lin et al., 2005; Persengiev et al., 2004;

Scacheri et al., 2004). Thorough studies of gene expression (*in vitro* and *in vivo*) with identification of the genes whose expression might be affected by specific miRNA techniques and with *in vivo* animal studies that identify potential unforeseen consequences are needed (Lewis, 2008). Such information potentially could lead to minimization of off-target effects through modification of experimental procedures. For example, small changes potentially could be made to the targeting sequence such that the desired mRNA continues to be targeted while targeting of other mRNAs is minimized (Jackson et al., 2006).

Use of techniques involving small regulatory RNAs such as miRNAs and siRNAs show great promise in the study of basic science questions as well as for therapeutic uses in a number of disorders including cancer. In the development and use of these techniques, safety concerns, particularly off-target effects and the consequences of greatly extending the life of small regulatory RNAs, should be considered in conducting risk assessments.

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Antimicrobial Testing Program Web Page Now Available

EPA has posted an Antimicrobial Testing Program web page to inform the public of post-registration efficacy test results of disinfectant products on the market for use in hospitals and other public health facilities. EPA conducts post-registration testing of public health antimicrobial products to ensure that marketed products are effective against target microorganisms when used according to their label directions. The ATP has been testing hospital sterilants, disinfectants, and tuberculocides since 1991 to help ensure that products in the marketplace continue to meet stringent efficacy standards. Products bearing claims to control organisms that may pose a threat to human health—either directly or through transmission of disease-causing organisms on environmental surfaces—are considered public health-related antimicrobials and require efficacy data to support labeling claims and patterns of use.

Under the ATP, Agency and state contract laboratories conduct tests to verify an antimicrobial product's effectiveness. The Agency has set the end of 2011 as the goal for completing the post-registration efficacy evaluation of the remaining hospital disinfectants and tuberculocides under the ATP. The web page at www.epa.gov/oppad001/antimicrobial-testing-program.html includes a list of products tested and their status. Updates to the product status list will be posted to the web site on a regular basis.