The Meaning of 21 in the MicroRNA World: Perfection Rather than Destruction?

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In this issue of *Cancer Cell,* Hatley et al., by using several tour-de-force in vivo approaches, reported a *miR-21*-mediated oncogenic pathway through inhibition of negative regulators of the Ras/MEK/ERK pathway and inhibition of apoptosis in lung cancers models. Targeting *miR-21* could be a promising therapeutic strategy in lung cancers.

Understanding MicroRNAs—from Eppendorf's Tubes to Mice and Finally to Humans

The discovery at the beginning of this decade that the few previously cloned tiny, worms-related short hairpin RNAs named microRNAs (miRNAs) are actually members of a very large class of genes expressed in the majority of metazoa organisms and important in any type of biological process took the scientific community by surprise (Ambros, 2008). The knowledge about miRNA functions and abnormalities accumulated since could create a mi-Revolution in the way the diagnosis, prognosis, and therapy for common diseases, including cancer, will be performed in the near future. MicroRNA expression profiling of human tumors has identified signatures associated with initiation and progression, diagnosis, staging, prognosis, and response to treatment (Croce, 2009). Most of the knowledge was obtained from studies performed in vitro in cell lines or in bulk tissue from patients, and therefore, what is needed actually are strong in vivo evidences that miRNAs are involved in signaling pathways important in normal cell homeostasis that are significantly deregulated in human diseases. The miRNAs to be exploited for the development of useful clinical markers and of new miRNA-based cancer therapies will be the ones proved to have a causal role.

MiR-21 Is the Most Significantly Overexpressed miRNA in Solid Cancers, including Lung

The research reported by Olson's group in this issue is important for several reasons (Hatley et al., 2010). First, it addresses a significant health problem. Despite the rapidly development of tumor markers and therapeutic agents, non-small-cell lung cancer (NSCLC), which account for ~80% of lung cancers, is the major leading cause of cancer-related deaths and the median survival of patients is still less than 1 year. There is, therefore, a clear need for early detection and identification of therapeutic targets and easy-to-test prognostic markers to optimize and personalize the diagnosis and treatment of lung cancer.

Second, the authors targeted the most significantly deregulated miRNA in human cancers. In the largest report on miRNA expression in human cancers, Volinia and colleagues identified *miR-21* as the most differentially expressed miRNA in 31 types of solid cancers by comparing 2532 cancer samples versus 806 corresponding normals (Volinia et al., 2010). This is not a simple quantitative finding, given that *miR-21* overexpression in NSCLC was associated with tumor aggressiveness and overall survival (Yanaihara et al., 2006).

Finally and importantly, despite the large amount of published data in human samples, all the previous reports showing the oncogenic activity of *miR-21* including high proliferation, low apoptosis, and high invasion and metastasis potential have been limited to in vitro assay. The report by Hatley et al. (2010) revealed for the first time a *miR-21* oncogenic pathway in vivo by using gain-of-function transgenic mice and loss-of-function knockout mice of *miR-21* allele in combination with the K-ras^{LA2} mouse model of NSCLC (CAG-*miR-21*; K-ras^{LA2} and the *miR-21*$^{-/-}$; K-ras^{LA2}, respectively). This was a wisely selected combination of crossings that pay back in a great way the hard work required for performing these experiments.

An In Vivo Autoregulatory Loop between Two Oncogenes—*miR-21* and RAS

The main finding, although not a great surprise at this point, was that *miR-21* act as a tumor promoter. *miR-21* overexpression in the CAG-*miR-21*; K-ras^{LA2} mice enhanced the incidence of all tumor grades without an increase in the rate of conversion from adenoma to adenocarcinoma, whereas *miR-21* deletion in the *miR-21*$^{-/-}$; K-ras^{LA2} mice suppressed formation of all stages of lung tumorigenesis, reducing the number of hyperplastic lesions and adenomas with no adenocarcinomas occurrence.

Another important finding is related to the identification of a multiplayer molecular network involved in proliferation and apoptosis (Figure 1). Previously published studies demonstrated that a number of targets for *miR-21* are tumor suppressors including TPM1, PDCD4, and PTEN. In addition, other targets including maspin, the apoptosis regulator BCL-2, the antiproliferative BTG2, and the sprouty homolog genes SPRY1 and SPRY2 have been validated mainly in vitro (for a review, see Krichevsky and Gabriely, 2009). The authors hypothesized that increased *miR-21* enhanced the Ras signaling pathway by inhibiting antagonists of the Ras/MEK/ERK pathway. They confirmed that tumors from CAG-*miR-21*; K-ras^{LA2} mice have decreased expression of Spry1, Spry2, and Btg2 protein compared to K-ras^{LA2} tumors resulting in the

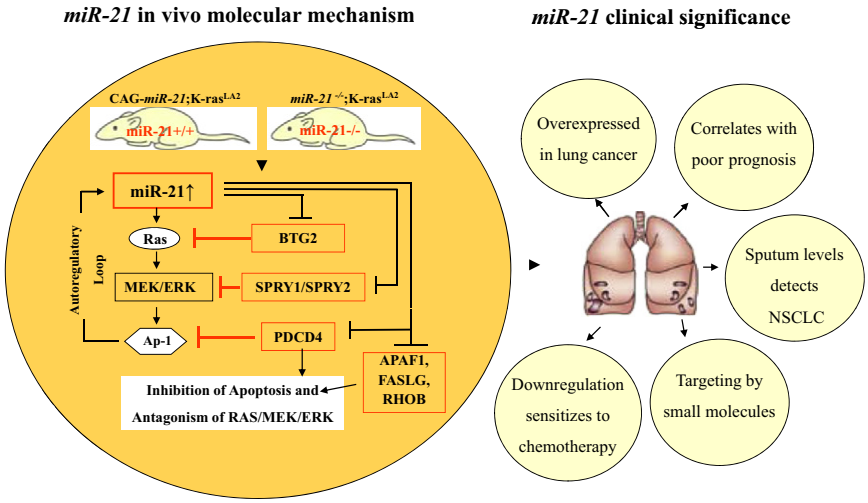

Figure 1. The Molecular Mechanism Involving *miR-21* and the Clinical Significance of *miR-21* Overexpression in Lung Cancers

The left panel represents the complex mechanism reported by Hatley and colleagues ("the perfection" of *miR-21* function), whereas the right panel indicates the main clinical applications (that transform *miR-21* "destructive" potential to a clinically useful potential). The gene names are as provided in the NCBI database at http://www.ncbi.nlm.nih.gov/gene.

activation of the Ras/MEK/ERK pathway. The authors also evaluated the target for *miR-21* involved in apoptosis and found that *miR-21* overexpression in CAG-*miR-21*; K-ras^{LA2} mice reduced apoptosis. The targets involved in apoptosis including Apaf1, Pdcd4, RhoB, and the Fas ligand Faslg in tumors from CAG-*miR-21*; K-ras^{LA2} mice were decreased. A take-home message from this research is that not all the targets shown by in vitro experiments have the same relevance in vivo. Good ways to identify such relevant interaction are either the use of mouse models or the identification of negative expression correlations in clinical samples with high purity of tumor cells.

Future Perspectives—Therapeutic Targeting of *miR-21*

At the center of a very complicated molecular network, *miR-21* can be exploited for therapeutic purposes. The authors initiated this investigation by discovering the fact that *miR-21* deletion can sensitize cells to DNA-damaging chemotherapy and its overexpression reduces consequent apoptosis. This report comes just in time, as Frank Slack's group reported on the use of Cre and Tet-off technologies to generate transgenic mice conditionally expressing *miR-21* (Medina et al., 2010). These mice developed a pre-B cell lymphoid-like phenotype and, importantly, tumors regressed completely in a few days when *miR-21* was suppressed. Such results suggest not only that that *miR-21* has genuine oncogenic activity but also that due to oncomiR addiction, suppression of this activity is enough for a potential therapeutic intervention.

Questions, Questions, Questions …

As all good science, the research by Olson group raises intriguing questions. For example, why the global expression of *miR-21* did not induce tumors, whereas conditionally overexpression does? The first in vivo proof that a single miRNA can cause cancer came from a B cell-specific *miR-155* transgenic mouse that developed B cell lymphoproliferative disease shortly after birth (reproducing the phenotype of the human leukemias where *miR-155* is highly expressed) generated by Croce's group (Costinean et al., 2006). Therefore, apparently the tissue-specific expression of miRNAs is the way to induce directly tumor formation. Also, is the reduced incidence of thymic lymphoma the only explanation why the survival of the CAG-*miR-21*; K-ras^{LA2} mice was not significantly decreased in spite of significantly increase in the tumors number (and consequently total tumor area) when compared with the K-ras^{LA2} mice? Did other unknown yet genetic elements (maybe other tissue-restricted noncoding RNAs or other regulatory elements) influence the tissue expression and natural history and subsequently survival? Last but not least, how should the therapeutic downregulation of *miR-21* be achieved? Antagomirs and other nucleic acids-based RNA inhibition approaches were reported. Another option not fully explored yet could be the SMIRs (small molecules that targets miRNAs) (Zhang et al., 2010). The advantage for using SMIRs is the availability of toxicity and the biodistribution studies in large primates that were already done for many of the small molecules in the past. Therefore the path to clinical application is shorter if a specific miRNA target is found. The first experimentally proof for this come exactly from a *miR-21* study, as Gumireddy and colleagues identified diazobenzene and its derivates as effective inhibitors of *miR-21* (Gumireddy et al., 2008). On the basis of the in vivo study of Olson's group, successful inhibition of *miR-21* by innovative drug therapies in NSCLC patients is several steps closer now and this is great news for both clinicians and patients!

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