A novel miRNA-mediated STOP sign in lung cancer: miR-340 inhibits the proliferation of lung cancer cells through $p27^{KIP1}$

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Abbreviations: CDK, cyclin-dependent kinase; CDKN1B, cyclin-dependent kinase inhibitor 1B ($p27$, Kip1); NSCLC, non-small cell lung cancer; PUM1/2, pumilio RNA-binding family member 1/2; SKP2, S-phase kinase-associated protein 2, E3 ubiquitin ligase.

Oncosuppressor miRNAs inhibit cancer cell proliferation by targeting key components of the cell cycle machinery. In our recent report we showed that miR-340 is a novel tumor suppressor in non-small cell lung cancer. miR-340 inhibits neoplastic cell proliferation and induces $p27^{KIP1}$ by targeting multiple translational and post-translational regulators of this cyclin-dependent kinase inhibitor.

Oncosuppressor miRNAs have emerged as powerful post-transcriptional inhibitors of genetic programs controlling cancer cell proliferation, survival, invasion, metastasis, and stemness. Moreover, the in vivo inhibition of tumor growth achieved in mouse cancer models by re-expression of well-characterized tumor suppressor miRNAs, such as miR-34 and let-7, suggests strong therapeutic potential.

Experimentally validated bioinformatics analyses show that cell cycle components are highly enriched among targets of the major tumor suppressor miRNAs. Several G1- and S-phase cyclins (D1, D3, and E2) and cyclin-dependent kinases (CDK4 and CDK6) represent key targets of let-7, miR-15/16, and miR-34 families. Conversely, several oncomiRs target the expression of CDK inhibitors; for example members of the miR-17–92 and miR-221/222 clusters target the $p27^{KIP1}$ transcript.

The CDK inhibitor $p27^{KIP1}$ is lost or inactivated in cancer cells by multiple mechanisms, including decreased synthesis, increased proteolysis, and mislocalization. The $p27^{KIP1}$ and $p57^{KIP2}$ transcripts are critical targets of the closely related miR-221 and miR-222 oncomiRs, which are overexpressed in multiple solid tumors including non-small cell lung cancer (NSCLC).

Downregulation of miR-340 has been reported in multiple tumors such as breast, colon, neuroblastoma, and osteosarcoma, in which miR-340 expression positively correlates with better prognosis. Experimentally validated miR-340 targets include disparate cellular components such as the tyrosine kinase MET in breast cancer, the transcription factors SOX2 in neuroblastoma and MITF in melanoma, and the cytoskeletal regulator ROCK1 in osteosarcoma.

We recently characterized miR-340 as a novel tumor suppressor in lung cancer and glioblastoma. miR-340 expression inversely correlates with clinical staging in NSCLC patients, whereas exogenous miR-340 inhibits proliferation and survival in NSCLC-derived cells. miR-340–induced growth arrest correlates with $p27^{KIP1}$ accumulation in both lung adenocarcinoma and glioblastoma cells. In A549 cells miR-340 controls $p27^{KIP1}$ at both translational and post-translational levels by directly targeting 3 negative regulators of $p27^{KIP1}$ (PUM1, PUM2, and SKP2) (Fig. 1). Human PUM1 and PUM2 genes encode 2 evolutionary conserved RNA-binding proteins related to the Pumilio gene products in *Drosophila* and *C. elegans*. The functional role of the PUM1/2 binding sites has been characterized for only a few human transcripts. Binding of PUM1 to the $p27^{KIP1}$ 3′-UTR induces a conformational switch that positively controls miR-221/222 accessibility. PUM2 has been suggested to act redundantly with PUM1. Consequently, $p27^{KIP1}$ expression is affected by PUM1/2 expression levels. Growth factor-induced phosphorylation of PUM1 Ser714 increases its RNA-binding activity, suggesting a role of PUM1 post-translational modification in the control of cell cycle re-entry.

The PUM1 and PUM2 transcripts share miR-340 target elements in their otherwise divergent 3′-UTRs. Our results show that miRNA-mediated downregulation of PUM1 and PUM2 antagonizes the
miR-221/222–mediated inhibition of p27KIP1. Remarkably, transcriptome-wide analyses of PUM1- and PUM2-bound mRNAs show significant enrichment for multiple cell cycle regulators in addition to p27KIP1. Therefore, the miR-340–PUM1/2 axis might control cell cycle progression by targeting multiple transcripts in addition to CDKN1B, which encodes p27KIP1.7 For example, PUM1/2 have also been implicated in the miRNA-mediated control of the cell cycle regulator E2F3 in bladder cancer cells.8

PUM1 belongs to a growing list of RNA-binding proteins including HuR, Dnd1, CRD-BP, and PTB that are implicated in the modulation of miRNA targeting in mammalian cells. Interestingly, the miR-340 target site in the MITF 3’-UTR is controlled by the CRD-BP RNA-binding protein, which interferes with miR-340 binding thus protecting the MITF transcript from miR-340–mediated degradation.9 Intriguingly, in addition to PUM1/2, miR-340 also targets 2 distinct RNA-binding proteins, PBP1/hnRNPA2, and RNA-binding proteins in cancer.9 p27KIP1 levels largely depend on protein stability, which is reduced by SCF^SKP2^-mediated ubiquitylation. Through investigation of the mechanism of p27KIP1 stabilization in miR-340-overexpressing cells we have identified S-phase kinase-associated protein 2, E3 ubiquitin ligase (SKP2), the substrate-recognizing component of the SCF^SKP2^-complex, as a target of miR-340. To our knowledge, this is the first evidence of miRNA-mediated regulation of the human SKP2 oncoprotein. In summary, miR-340 could influence G1/S transition by affecting the accumulation of cyclins D1/D2 and the activity of cyclin D/CDK4/6 complexes, together with the induction of p27KIP1 (via PUM1/2 and SKP2) and inhibition of the cyclin E/CDK2 complex. In addition, having observed that miR-340 is responsive to serum induction we postulate that miR-340 might participate in the control of cell cycle progression in response to extracellular mitogenic signals.

In addition to further studies aimed at the transcriptome-wide identification of target mRNAs and oncogenic networks modulated by miR-340, future investigations will address the applications of miR-340. Importantly, systemic delivery of pre-miR-340 has recently been shown to inhibit the growth of xenografts of human colorectal cancer cells in mice.10 Therefore, multiple lines of evidence point to miR-340 as a novel, highly promising bullet for miRNA-based anticancer therapeutics.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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