COMMENTARY

A primate-specific microRNA enters the lung cancer landscape

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Characterization of the lung cancer genome through unbiased next-generation sequencing has led to the discovery of new driver somatic mutations (1, 2) and gene fusions (3). Lung cancers show the second highest prevalence of somatic mutations among all cancer types, and a mutation profile that reflects the effect of chronic exposure to tobacco carcinogens, the main risk factor etiologically associated with this disease (2–4). The next chapter of genomic decoding, through small RNA sequencing, brings about the discovery of primate-specific microRNAs (miRNAs) of the airway epithelium and the intriguing possibility that some may function as lung tumor suppressors (5).

miRNAs were first discovered and investigated in 1993 by Ambros and colleagues and Ruvkun and colleagues (6, 7) in the course of studies on developmental timing of *Caenorhabditis elegans*. Despite their recent historical account, miRNA-mediated post-transcriptional control of protein-coding genes is an evolutionarily ancient mechanism that regulates key developmental processes in eukaryotes. With the degree of granularity afforded by deep sequencing technologies, the number of expressed sequences showing evidence of a characteristic miRNA hairpin structure keeps rising. The 20th edition of miRBase, the catalog of miRNA sequences, released in June 2013, contains more than 24,000 entries for hairpin precursor miRNAs. Surprisingly, with more miRNA sequences revealed comes the realization that an astounding nearly 30% of them are unique to primates, and even a few, such as miR-941, may be unique to humans (8). They are thought to underlie phenotypic variation between species, possibly at the foundation of

unique human traits. Only a handful of primate-specific miRNAs have been shown to be properly processed, functionally characterized, and associated with a phenotype or signaling pathway. Among them, primate-specific miR-198 participates in a regulatory loop that prevents wound healing, a process not readily associated with a higher evolutionary trait (9). In PNAS, Perdomo et al. (5) identify miR-4423 as a primate-specific miRNA expressed primarily in ciliated cells of the airway epithelium. Upon demonstrating that miR-4423 follows standard miRNA biogenesis, they cleverly dissect its function in normal tissue through the use of specialized culture techniques, reasserting the exquisite cell type and context specificity that is a hallmark of miRNA regulation. Expression of miR-4423 could only be observed in vitro when bronchial epithelial cells were differentiated into mucociliary epithelia at an air–liquid interface.

Loss of expression of tissue-specific miRNAs has been implicated in human cancer, due to genetic deletion (10) and epigenetic silencing (11) characteristic of tumor suppressor genes. Two primate-specific miRNAs, miR-637 and miR-520e, are down-regulated in human hepatocellular carcinoma and exhibit properties of tumor suppressors, including inhibition of xenograft growth (12, 13). Similarly, lower expression of miR-4423 was found in the bronchial epithelium of smokers with lung cancer and in tumor tissue, and its enforced expression modestly reduced anchorage-dependent in vitro growth in a subset of lung cancer cell lines and reduced the size of xenografts generated by inoculation of a lung squamous cell carcinoma cell line into immunocompromised mice (5). The morphological, immunohistochemical, and gene expression changes associated with miR-4423 overexpression appear to be consistent with activation of a differentiation program in bronchial epithelial cells and prompt Perdomo et al. (5) to speculate that miR-4423 may be
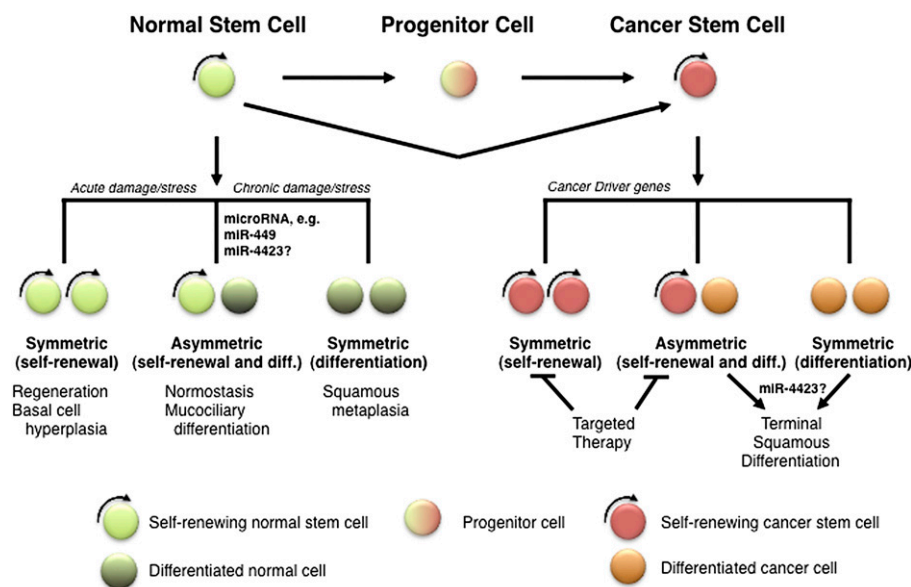

Fig. 1. Normal stem cells respond to either acute or chronic stress and damage by regeneration or terminal differentiation, respectively. Cancer driver genes may promote tumorigenesis in part by shifting the balance of renewing symmetric and asymmetric cell divisions. Current therapeutic approaches fail to eradicate cancer stem cells (CSCs), leading to recurrence and metastasis. Modulation of symmetric division of CSC through targeting of cancer driver genes, e.g., Alk fusions, CSC-specific pathways, and of asymmetric division by inducing terminal squamous differentiation, e.g., miR-4423 as proposed by Perdomo et al. (5), or a combination of both may provide an opportunity for the development of innovative therapeutic strategies.

a candidate for redifferentiation of cancer stem cells as proposed by Sell more than four decades ago (14) and here illustrated in Fig. 1. The implication that miR-4423 may be a driver of lineage differentiation in the small airways suggests a possible therapeutic window for a miR-4423 mimic to restore a normal differentiation pattern during the early steps of tumorigenesis and may even have cancer preventive value. However, knockdown of miR-4423 did not greatly affect ciliated differentiation of bronchial epithelial cells, implying its functional redundancy with other factors, possibly other miRNAs, like miR-449/miR-34 family members with whom miR-4423 shares partial seed sequences and predicted targets. miR-449 accumulates when bronchial epithelial cells are differentiated into mucociliary epithelia at an air–liquid interface (15) and controls ciliated differentiation and cell fate (16, 17). Expression of miR-34 is frequently reduced in cancers, and its reexpression has pleiotropic effects on cancer cell phenotypes, including inhibition of cancer stemness (18). A first-in-kind phase I clinical trial to evaluate safety of miR-34 replacement therapy was started earlier this year (19).

The interplay of miR-4423 with its proximal gene *WDR63* (WD repeat domain 63) brings an interesting scenario. The precursor and mature forms of miR-4423 are coexpressed with WDR63 in mucociliary epithelium and similarly induced when bronchial epithelial cells are differentiated into mucociliary epithelia at an air–liquid interface, suggesting transcription from a bicistronic locus. WDR63 is also down-regulated in lung cancers, possibly through DNA methylation. But, unlike miR-4423, WDR63 is highly conserved beyond primates, and serves a structural function in cilia. It is unclear whether DNA methylation may influence transcription at both loci. Similarly, DNA damage and stress-induced transcription factors may concomitantly affect miR-4423 and WDR63.

Smoking exerts toxic, epigenetic, and mutational effects on the airway epithelium (20), promoting a progressive loss of ciliated cells, which would in itself lead to the observed

Perdomo et al. identify miR-4423 as a primate-specific miRNA expressed primarily in ciliated cells of the airway epithelium.

decrease in expression of miR-4423. Under those circumstances, the value of miR-4423 as a lung cancer biomarker becomes less clear. Does the decrease in expression of miR-4423 indeed drive dedifferentiation of the mucociliary epithelium or is it secondary to the loss of ciliated epithelium that accompanies smoking? A more detailed investigation of miR-4423 expression in the progression from normal bronchial epithelium, squamous metaplasia, dysplasia to carcinoma in situ may shed valuable insight on its function. In addition, further studies aimed at delineating nonredundant functions of miR-4423 are needed. It is possible that supraphysiological levels of miR-4423 achieved by ectopic expression could functionally replace miR-449 rather than reflect a specific role of miR-4423. Therefore, reliable knockdown experiments will be required to elucidate the unique function of miR-4423. Lack of sequence conservation beyond primates will prevent the use of straightforward knockout strategies in murine animal models. Human organoid studies in vitro and the use of primary tumor explants in immunosuppressed mice may provide avenues for dissection of the role of miR-4423 in lung carcinogenesis.

1 Pao W, Girard N (2011) New driver mutations in non-small-cell lung cancer. *Lancet Oncol* 12(2):175–180.
2 Alexandrov LB, et al.; Australian Pancreatic Cancer Genome Initiative; ICGC Breast Cancer Consortium; ICGC MMML-Seq Consortium; ICGC PedBrain (2013) Signatures of mutational processes in human cancer. *Nature* 500(7463):415–421.
3 Govindan R, et al. (2012) Genomic landscape of non-small cell lung cancer in smokers and never-smokers. *Cell* 150(6):1121–1134.
4 Imielinski M, et al. (2012) Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. *Cell* 150(6): 1107–1120.
5 Perdomo C, et al. (2013) MicroRNA 4423 is a primate-specific regulator of airway epithelial cell differentiation and lung carcinogenesis. *Proc Natl Acad Sci USA*, 10.1073/pnas.1220319110.
6 Lee RC, Feinbaum RL, Ambros V (1993) The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 75(5):843–854.
7 Wightman B, Ha I, Ruvkun G (1993) Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. *Cell* 75(5):855–862.
8 Hu HY, et al. (2012) Evolution of the human-specific microRNA miR-941. *Nat Commun* 3:1145.
9 Sundaram GM, et al. (2013) 'See-saw' expression of microRNA-198 and FSTL1 from a single transcript in wound healing. *Nature* 495(7439):103–106.
10 Calin GA, et al. (2002) Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 99(24):15524–15529.

11 Lopez-Serra P, Esteller M (2012) DNA methylation-associated silencing of tumor-suppressor microRNAs in cancer. *Oncogene* 31(13):1609–1622.
12 Zhang JF, et al. (2011) Primate-specific microRNA-637 inhibits tumorigenesis in hepatocellular carcinoma by disrupting signal transducer and activator of transcription 3 signaling. *Hepatology* 54(6):2137–2148.
13 Zhang S, et al. (2012) MicroRNA-520e suppresses growth of hepatoma cells by targeting the NF-κB-inducing kinase (NIK). *Oncogene* 31(31):3607–3620.
14 Sell S (1993) Cellular origin of cancer: Dedifferentiation or stem cell maturation arrest? *Environ Health Perspect* 101 (Suppl 5):15–26.
15 Martinez-Anton A, et al. (2013) Changes in microRNA and mRNA expression with differentiation of human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 49(3):384–395.
16 Marcet B, et al. (2011) Control of vertebrate multiciliogenesis by miR-449 through direct repression of the Delta/Notch pathway. *Nat Cell Biol* 13(6):693–699.
17 Lizé M, Klimke A, Dobbelstein M (2011) MicroRNA-449 in cell fate determination. *Cell Cycle* 10(17):2874–2882.
18 Bu P, et al. (2013) A microRNA miR-34a-regulated bimodal switch targets notch in colon cancer stem cells. *Cell Stem Cell* 12(5): 602–615.
19 Bader AG (2012) miR-34: A microRNA replacement therapy is headed to the clinic. *Front Genet* 3:120.
20 Wistuba II, Mao L, Gazdar AF (2002) Smoking molecular damage in bronchial epithelium. *Oncogene* 21(48):7298–7306.