

Secreted microRNAs from tumor cells can suppress immune function

Xi Chen[‡], Hongwei Liang[‡], Ke Zen^{*}, and Chen-Yu Zhang^{*}

Jiangsu Engineering Research Center for MicroRNA Biology and Biotechnology (JERC-MBB); State Key Laboratory of Pharmaceutical Biotechnology; School of Life Sciences; Nanjing University; Nanjing, Jiangsu China

[‡]These authors contributed equally to this work.

Keywords: microvesicle, *PTEN*, regulatory T cell, secreted microRNA, tumor immune escape

Our recent study reported an interesting finding that tumor cells can actively manipulate host immune function to facilitate tumor immune escape through the secretion of microRNAs (miRNAs). As an extension of this finding, we showed that blockage of the function of tumor-secreted miRNAs represents an effective therapeutic approach for cancer treatment.

The intensive studies of the past several years have documented the importance of miRNAs as an essential cornerstone of the genetic system.¹ Although RNA is usually considered an unstable molecule because of the ubiquitous RNase, miRNAs are now known to circulate in the bloodstream and other body fluids in a stable, cell-free form.^{2,3} Importantly, we and other groups have demonstrated that a large proportion of extracellular miRNAs are actively secreted from host cells through a pathway based on small membranous vesicles known as microvesicles.^{4,5} Under the protection of microvesicles, secreted miRNAs can be delivered to recipient cells, where they block the translation of their target genes and regulate recipient cell function.^{4,5} Thus, secreted miRNAs can serve as a novel class of signaling molecules in mediating intercellular communication.

In a newest study,⁶ we tested the hypothesis, if tumor cells can actively secrete miRNAs to exert a suppressive effect on immune cells, particularly regulatory T cells (Tregs), a subset of CD4⁺ T cells that plays an important role in maintaining self-tolerance and modulating immune responses. We first identified miR-214 as a major oncogenic miRNA secreted by various types of cancers through microvesicles. Secreted miR-214

then enters recipient CD4⁺ T cells, in which the exogenous miR-214 decreases the expression of its target gene, *phosphatase and tensin homolog (PTEN)*. Since *PTEN* is a negative modulator of Treg homeostasis *in vivo* and expansion *ex vivo*,⁷ downregulation of *PTEN* by tumor-secreted miR-214 results in an expansion of Tregs. Finally, as Tregs are increased during tumorigenesis and efficiently induce tumor immune evasion,⁸ Treg expansion mediated by secreted miR-214 leads to host immune tolerance, therefore causing rapid growth of tumor cells. Taken together, this study reveals a novel mechanism through which tumor cell actively modulate the host immune system via the secretion of miRNAs and induction of Treg expansion (Fig. 1).

This study reveals some thought-provoking points. Previously, it is believed that tumor cells utilize two mechanisms to evade the immune response: (a) modification of the tumor cells themselves to decrease their sensitivity to attacks by functional immune cells, and (b) direct modulation of the host immune cells by the secretion of cytokines. This study raises a novel standpoint that tumor cells can actively manipulate the antitumor activities of immune cells by delivering tumor-specific miRNAs to immune cells. This study also suggests that: (a) although the

levels of secreted miRNAs in extracellular space is quite low, they have the full capacity to exert essential biological functions; (b) secreted miRNAs may represent a new defining hallmark of the tumor microenvironment; (c) secreted miRNAs may play a pivotal role in tumor metastasis.

This finding may also have far-reaching clinical applications. Therapeutics targeting tumor immune escape is a promising approach for cancer treatment. Because secretion of miRNAs likely serves as a common mechanism for various cancer cells to create a tolerant immune environment, blocking the function of tumor-secreted miRNAs is, in principle, a feasible strategy to reverse tumor-induced immune tolerance. By employing cell-derived microvesicles to deliver miR-214 antisense (a strand of oligonucleotide complementary to miR-214) into peripheral CD4⁺ T cells, we successfully blocked Treg expansion and tumor growth induced by tumor cell-secreted miR-214.⁶ This revolutionary approach may fill the void left by current methods for systemic gene therapy. Currently, we are making great efforts to improve and validate the effectiveness of this approach in cancer therapy.

The exciting research field of miRNA secretion is still in its infancy stage. In the present study, we reported a new signaling

*Correspondence to: Chen-Yu Zhang; Email: cyzhang@nju.edu.cn; Ke Zen; Email: kzen@nju.edu.cn

Submitted: 10/25/2014; Accepted: 10/28/2014

<http://dx.doi.org/10.4161/2162402X.2014.982407>

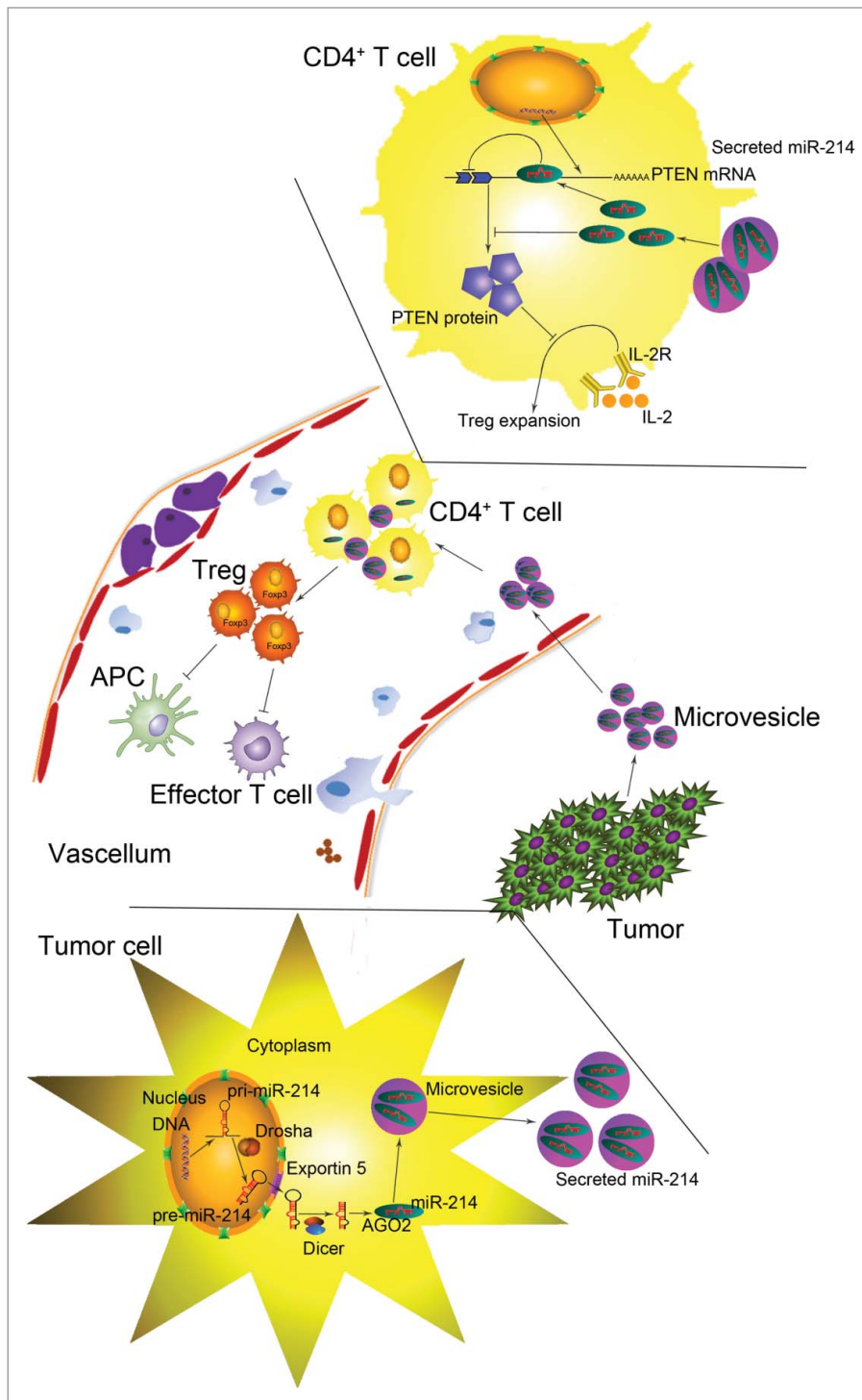


Figure 1. Working model for the role of tumor-secreted miR-214 in inducing tumor immune escape. After being processed into mature miRNA, tumor-specific miR-214 is packaged into microvesicles and secreted to the extracellular environment by tumor cells. Secreted miR-214 is then delivered by microvesicles to the recipient CD4⁺ T cells, in which it decreases PTEN protein expression and facilitates Treg expansion. Increased Treg population, in turn, leads to host immune suppression and tumor growth.

pathway between tumor and immune cells. Interestingly, some recent studies have also shown the active participation of tumor-secreted miRNAs in cancer

progression and metastatic spread.^{9,10} These studies reveal the importance of tumor-secreted miRNAs as a novel and critical cancer hallmark. However, some

outstanding questions remain to be addressed. For example, extensive investigation is required to determine how oncogenic miRNAs are specifically targeted for

secretion and are recognized for uptake. It is also largely unknown regarding the value of tumor-secreted miRNAs in cancer

treatment. We anticipate that future studies in this area will revolutionize the way we perceive how tumor cells communicate

with immune cells and open up new avenues for wide-spread and efficient treatments against cancer.

References

1. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; 116:281-97; PMID:14744438; [http://dx.doi.org/10.1016/S0092-8674\(04\)00045-5](http://dx.doi.org/10.1016/S0092-8674(04)00045-5)
2. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008; 18:997-1006; PMID:18766170; <http://dx.doi.org/10.1038/cr.2008.282>
3. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL et al. Circulating microRNAs as stable blood-based markers for cancer detection. *P Natl Acad Sci* 2008; 105:10513-8; PMID:18663219; <http://dx.doi.org/10.1073/pnas.0804549105>
4. Zhang YJ, Liu DQ, Chen X, Li J, Li LM, Bian Z, Sun F, Lu J, Yin Y, Cai X et al. Secreted monocytic miR-150 enhances targeted endothelial cell migration. *Mol Cell* 2010; 39:133-44; PMID:20603081; <http://dx.doi.org/10.1016/j.molcel.2010.06.010>
5. Kosaka N, Iguchi H, Yoshioka Y, Takeshita F, Matsuki Y, Ochiya T. Secretory mechanisms and intercellular transfer of microRNAs in living cells. *J Biol Chem* 2010; 285:17442-52; PMID:20353945; <http://dx.doi.org/10.1074/jbc.M110.107821>
6. Yin Y, Cai X, Chen X, Liang H, Zhang Y, Li J, Wang Z, Chen X, Zhang W, Yokoyama S et al. Tumor-secreted miR-214 induces regulatory T cells: a major link between immune evasion and tumor growth. *Cell Res* 2014; 24:1164-80; PMID:25223704; <http://dx.doi.org/10.1038/cr.2014.121>
7. Walsh PT, Buckler JL, Zhang J, Gelman AE, Dalton NM, Taylor DK, Bensinger SJ, Hancock WW, Turka LA. PTEN inhibits IL-2 receptor-mediated expansion of CD4⁺ CD25⁺ Tregs. *J Clin Invest* 2006; 116:2521-31; PMID:16917540; <http://dx.doi.org/10.1172/JCI28057>
8. Savage PA, Malchow S, Leventhal DS. Basic principles of tumor-associated regulatory T cell biology. *Trends Immunol* 2013; 34:33-40; PMID:22999714; <http://dx.doi.org/10.1016/j.it.2012.08.005>
9. Zhou WY, Fong MY, Min YF, Somlo G, Liu L, Palomares MR, Yu Y, Chow A, O'Connor ST, Chin AR et al. Cancer-secreted miR-105 destroys vascular endothelial barriers to promote metastasis. *Cancer Cell* 2014; 25:501-15; PMID:24735924; <http://dx.doi.org/10.1016/j.ccr.2014.03.007>
10. He WA, Calore F, Londhe P, Canella A, Guttridge DC, Croce CM. Microvesicles containing miRNAs promote muscle cell death in cancer cachexia via TLR7. *P Natl Acad Sci* 2014; 111:4525-9; PMID:24616506; <http://dx.doi.org/10.1073/pnas.1402714111>