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REVIEW

Tumor-secreted microRNAs act as intercellular communication mediators to manipulate the host immune system

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Tumor cells influence their environment by releasing various substances such as cytokines and chemokines. Studies over the past few years have shown that microvesicles (MVs) secreted by tumor cells contain proteins, mRNAs, and microRNAs (miRNAs) that play important roles in intercellular crosstalk. Recently, we reported an exciting finding that tumor cells can manipulate the host immune response via secreting an important oncomir, miR-214, through a pathway involving MVs. As an extension of this finding, we showed that the delivery of miR-214 antisense oligonucleotides via MVs can significantly inhibit tumor-induced immune escape and tumor growth, which provides a novel, effective approach for tumor treatment.

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

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Tumor cell communication is a complex and dynamic process involving multiple mechanisms that allow tumor cells to manipulate their environment [1]. Tumors secrete a diversity of signaling molecules to control the behavior of immune cells, fibroblasts, vascular tissues, and surrounding extracellular matrix [2]. Among the factors contributing to tumorigenesis, the failure of the immune system to protect against tumor cells is one of the main factors by which tumors survive, grow, proliferate, and metastasize [3]. However, the detailed mechanism of tumor immune escape remains to be elucidated.

MicroRNAs (miRNAs) are a class of small noncoding RNA that are frequently dysregulated in cancers [4]. Once thought to only exist in intracellular space, miRNAs have now been demonstrated to be stably present in serum, urine, saliva, and other bodily fluids [5 ,6]. A significant amount of these miRNAs exist in extracellular environment in a form that is enclosed in small membranous vesicles named microvesicles (MVs, particularly exosomes and shedding vesicles) [7]. Through MV-cell interactions, MVs can actively deliver miRNAs to recipient cells, in which exogenous miRNAs can inhibit the expression of their target genes and manipulate recipient cell function. Thus, miRNAs secreted through MVs are active and can function as novel signaling molecules to mediate cell-to-cell communication [8].

In a recent study [9], we provided evidence indicating that tumor cells actively secrete miRNAs to exert a suppressive effect on immune cells, particularly regulatory T cells (Tregs), which is a subtype of $CD4^+$ T cells that plays a pivotal role in tumor immune escape. Specifically, we observed increased secretion ofmiR-214, an important oncogenic miRNA, in a variety of human cancers and mouse tumor models. Tumor-secreted miR-214 was sufficiently transfered to recipient $CD4^+$ T cells via MVs, and exogenous

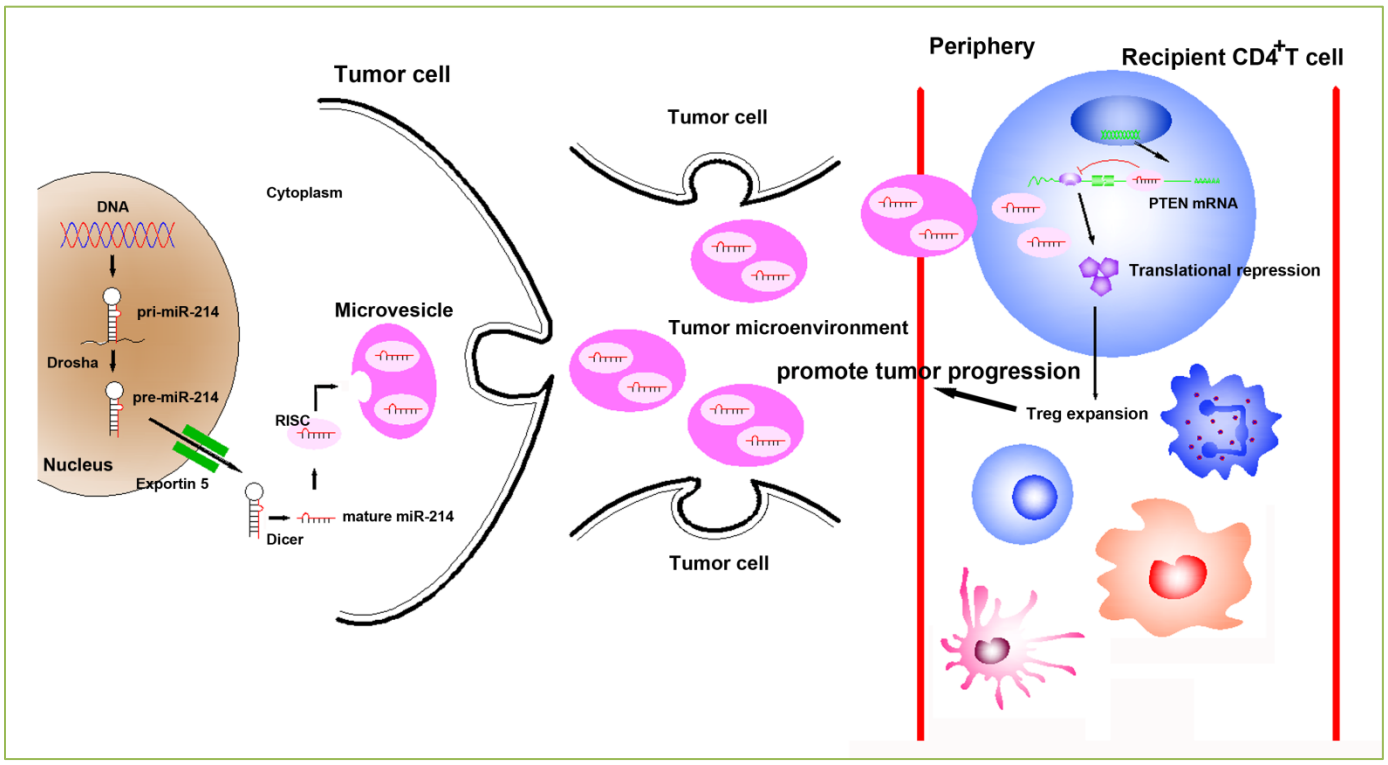

Figure 1. Working model of tumor-secreted miR-214 on the induction of Tregs. Tumor cells secrete miR-214 via MVs to the extracellular environment. Secreted miR-214 is then delivered into peripheral $CD4^+$ T cells. After the MVs enter the recipient $CD4^+$ T cells, miR-214 inhibits PTEN protein expression and facilitates Treg expansion, which, in turn, results in host immune suppression and tumor growth.

miR-214 downregulated its target gene, phosphatase and tensin homolog (PTEN), resulting in an expansion of functional Tregs, as PTEN is a negative regulatory factor of Treg homeostasis *in vivo* and Treg expansion *ex vivo* [10]. Because Tregs are potent inducers of tumor immune escape [11], the increased Treg population mediated by tumor-derived miR-214 led to host immune tolerance, therefore causing immune escape and rapid growth of the tumor cells (Figure 1).

The function of secreted miRNAs in tumorigenesis has only been investigated recently. Several studies have revealed that tumor-secreted miRNAs can participate in tumor progression by inducing tumor formation, promoting mesenchymal-to-epithelial transition, or destroying vascular endothelial barriers to promote metastasis [12, 13]. These studies focused on investigating the crosstalk between malignant cells and nontumorigenic epithelial cells or on strongly and weakly metastatic cancer cells. Our study raises a novel standpoint that tumor cells can actively manipulate the antitumor efficacy of immune cells and change the property of surrounding cells in the tumor microenvironment. Our thought-provoking work reveals the importance of tumor-secreted miRNA as a novel regulator of cancer immunology.

Because immune escape is always correlated with tumorigenesis, the modulation of the immune system for tumor therapy is an intensive area of research [14]. However, immune-based therapy is often difficult to apply in practice due to the organism's self-defense. Because MVs are natural nanocarriers derived from endogenous cells, they will be better tolerated by the immune system [15]. Furthermore, MVs are thought to be an ideal delivery system due to their small size and abilities to cross biological membranes and protect their cargo from degradation. By employing cell-derived MVs to deliver anti-miR-214 antisense oligonucleotides to peripheral $CD4^+$ T cells, we successfully reversed the downregulation of PTEN in $CD4^+$ T cells and repressed the upregulation of Treg population induced by tumor cells. As anticipated, the MV-delivered anti-miR-214 significantly decreased the growth of implanted and spontaneous tumors. Moreover, proteins that are indispensable for miRNA function, such as Argonaute 2 (AGO2) [16, 17], were found to be bound to miRNAs in MVs. Thus, MVs have the natural advantage to traverse biological barriers and transport functional miRNAs across cells. Therefore, the miRNA delivery system based on MV carrier may provide a novel method for gene therapy.

Taken together, our study demonstrates for the first time that tumor cells can actively regulate immune cell function via secreting tumor-specific miRNAs. This study

significantly extends the role of miRNAs and opens up new avenues for investigating the secreted miRNA-mediated interactions of tumor cells and immune cells. However, these interactions are still largely unknown, especially regarding the specificity and sufficiency of the secretion of the miRNA and its special recognition by target cells. Overall, more extensive investigations are required in the future to determine the application potential of secreted miRNAs as attractive molecules in tumor therapy.

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