**Tumor-secreted miR-214 delivered on T cells to promote tumor growth**

Key words: secreted microRNA; regulatory T cells; microvesicles; immune evasion, tumor

 **There is a mysterious link between increased population of CD4+CD25high Foxp3+ regulatory T cells (Tregs) in the tumor and cancer immune. This article gives a mechanism for the first time, in which tumor-secreted miR-214 is delivered to T cells by microvesicles (MVs), then downregulate phosphatase and tensin homolog (PTEN) and promote tumor growth. The MV delivery of anti-miR-214 antisense oligonucleotides (ASOs) efficiently blocks Treg expansion and suppress tumor growth.**

**Introduction**

During tumorigenesis, many substances in cells have abnormal activities, some of which are good signals of tumor and provide potential therapies for cancer.

What is known for sure is increased population of Tregs influences efficiently the host immune response and promote tumor growth. Various factors contributes to the expansion of Tregs, which is still under research.

On the other hand, microRNAs (miRNAs), a class of noncoding RNAs with lengths of approximately 22 nucleotides, take an important part in many gene regulation. Some abnormal miRNAs expression are observed in tumor. Furthermore, in certain tumor types, specific miRNAs can function as oncogenes or tumor suppressors. The connection between certain miRNAs and T cells or immune response is observed, while is not fully undertood.

In this research, several types of human cancers and mouse tumor models were observed and a hypothesis was came up, which later was proved to be right. Tumor-secreted miR-214 in microvesicles is delivered on Tregs to promote them to expand by downregulating PTEN. The increased population of Tregs enhances immune suppression. Meantime miR-214 induces Tregs to secret more IL-10 and promote tumor growth. MiRNA-214 secreted by tumor cells works as a signaling molecule in mediating cell-cell communication here.

**Recent Progress**

 Several steps were conducted to prove the hypothesis step by step.

1. miR-214 levels were higher in observed various cancers’ tissues and tumor implanted mice

Some miRNAs were considered oncogenic in certain tumors, and miR-18a, miR-19a, miR-220a, miR-21, miR-210, miR-214, and miR-92a were mainly observed here. After assessing their expression in the tissues from patients with breast cancer, hepatocellular carcinoma, non-small-cell lung cancer, or pancreatic cancer. It turned out only the level of miR-214 was significantly increased in all the cases observed. Besides in plasma, the levels of miR-214 were enriched in MVs.

1. Lewis Lung Cancer (LLC) cell-secreted miR-214 MVs promoted Tregs expansion

LLC-derived MVs containing a high level of miR-214 were incubated with primary CD4+T cells to prove secreted miR-214 was delivered into targeted Tregs efficiently. The increased miR-214 levels in T cells and unchanged levels of pre-miR-214 in T cells prove that miR-214 came mainly from secreted MVs instead of produced by T cells themselves.

As a comparison, miR-214-deficient LLC-derived MVs were prepared by knocking down miR-214 using antisense oligonucleotides (ASOs). It turned out miR-214-deficient MVs did not influence the level of miR-214 in T cells and failed to affect Treg expansion.

1. Decreased PTEN by LLC-secreted miR-214 resulted in Treg expansion

The less active PTEN promotes Treg expansion. Treatment with with LLC MV but not LLC MV/miR-214def repressed PTEN. Neither LLC MVor LLC MV/miR-214def influenced the mutant PTEN. No change was in PTEN mRNA level, which suggested miR-214 affected PTEN at the protein rather than mRNA level.

After introducing miR-214-resistant PTEN, Treg expansion was not observed, which suggested LLC-secreted miR-214 downregulated PTEN in CD4+ T cells, leading to Treg expansion.

1. LLC-secreted miR-214 induced IL-10 secretion in CD4+ T cells

LLC MVs just increased the levels of interleukin-10 (IL-10), not TGB-β or interleukin -12 (IL-12). And lack of miR-214 blocked IL-10 production. Therefore miR-214 might increase Treg population via IL-10, furthermore the immune system was suppressed.

1. Above results were confirmed in vivo

Through injection of fluorescent labeled LLC MVs and use of specific probes to track miR-214def, the transportation of miR-214 into CD4+ T cells in vivo were monitored. It showed that cancer-secreted miR-214 might be delivered into CD4+ T cells via MVs. No change in pre-miR-214 levels suggested the elevated miR-214 levels in T cells was not produced by T cells itself but secreted by cancer cell. Meantime, the population of CD4+CD25highFoxp3+ Tregs increased significantly, which compromised the immune response to the tumors, leading rapid tumor growth.

To prove miR-214 truly worked on Tregs in vivo, Treg-deficient mice were treated with tumor-derived MVs. The result was confirmed by that neither LLC MVs nor LLC MV/miR-214def promoted tumor growth. But Treg elimination in mice caused a more rapid development of terminal autoimmune disease, or worse, becoming moribund.

1. Blocking of cancer cell-secreted miR-214 downregulated Treg induction and reduced tumor growth

Considering the tumor-secreted miR-214 modulated Tregs, downregulation of circulating miR-214 levels could decrease the numbers of Tregs to suppress tumor growth. After treated with anti-miR-214 ASOs in tumor implanted mice, the miR-214 levels decreased, so did the levels of PTEN, and the expansion of CD4+CD25highFoxp3+ T cells was suppressed. Compared with the control mice, a significantly decreased growth rate and tumor size.

**Discussion**

This article reveals a new mechanism by which tumor cells use secreted-miRNA-214 as a regulator of Tregs to suppress immune system and promote tumor growth in turn. Secreted miRNAs in MVs could function as a new pathway of intercellular communication.

Previously although it is known increased Tregs promotes tumor growth, human Treg-based therapies are difficult to implement in the clinic, because blocking Tregs is so harmful in other ways. Now, inhibiting the transport of secreted miR-214 might work as a new strategy to stop the communication between cancer cells and immune systems.

Investigations on the biological functions of miRNAs are still in their infancy, and the role of secreted miRNAs in tumor progression has just been researched recently. Some connections between various miRNAs and their message recipients may be observed, but mechanisms are still not clear enough.

This article presents a good mechanism in how tumor-secreted miRNA functions in tumorigenesis by suppressing the immune response. Some other researches have shown tumor-secreted miRNAs can participate in tumor spread or promote muscle loss during tumorigenesis. With further work, it is promising to develop a novle and effective therapeutic approach for cancer treatment through secreted miRNAs.

**References**

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