MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response

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AUTHOR SUMMARY

MicroRNAs (miRNAs) are small noncoding RNAs possessing gene-regulatory functions and are secreted by cancer cells by means of microvesicles called “exosomes.” Here we show that miRNAs contained in cancer cell-secreted exosomes can be transferred to surrounding immune cells and can bind to Toll-like receptors (TLR7 in mice and TLR8 in human). By binding to TLRs, miRNAs induce cytokine secretion by the immune cells, leading to a prometastatic inflammatory response (Fig. P1). Here, we report that miRNAs can act as hormones by binding to receptors.

MiRNAs are expressed aberrantly in most types of cancer (1, 2). They are secreted from cells within exosomes and are transferred from cell to cell; miRNAs regulate gene expression in the recipient cells by binding to their target messenger RNAs (3). MiRNAs also can interact with proteins (4). For example, members of the TLR family (murine TLR7 and human TLR8) bind viral single-stranded RNA sequences on immune cells, leading to cytokine production (5). Circulating miRNAs could represent ligands of TLR7 and TLR8 that are released by tumor cells and are involved in intercellular communication in the tumor microenvironment.

We first determined which miRNAs are represented more frequently in the exosomes secreted by different populations of lung cancer cell lines, using NanoString technology and subsequent validation by quantitative real-time PCR. We found that miR-21, -29a, and -16 were among the most represented in cancer cell-secreted exosomes. Subsequently, we used immunoprecipitation techniques to show that these three miRNAs are able to reach and bind to TLR8 in the endosomes of a cell. Using in situ hybridization with the Nuance system, we were able to demonstrate in primary human lung cancer tumors that cancer cells are the main producers of miR-29a secreted in the exosomes and that miR-29a colocalizes with macrophages, mainly at the interface of tumor and normal tissue. Next, we asked whether the binding of miRNAs to TLRs resulted in activation of the TLR-mediated signaling pathway. We observed that although miR-21 and -29a are able to activate a TLR8-mediated response (activation of the NF-κB pathway and secretion of TNF-α and IL-6), miR-16 does not. We performed an initial analysis on whether this difference might be explained by the sequence of the mature miRNAs by performing mutagenesis of specific nucleotides in the mature sequence of the miRNAs of interest. Although we showed that some nucleotides are critical for the activation of TLRs, further studies are warranted to clarify this aspect. We also showed that the binding of miR-21 and -29a to TLR7 and TLR8 induces activation of immune cells (indicated by activation of CD69) and that this effect is not mediated by binding to human TLR7. Moreover, we showed in an in vivo mouse model of lung cancer [mice injected with Lewis Lung Carcinoma (LLC) cells] that, by binding to TLR7, cancer cell-secreted miR-21 and -29a in exosomes metastasize preferentially to the lung and reduce overall survival, compared with TLR knockout mice in which this mechanism is impaired. We also found that drugs affecting exosome secretion by cancer cells significantly reduce the metastatic potential of LLC cells, and this effect can be rescued by injecting tumor-bearing mice with exosomes secreted by LLC cells.

Our finding here that miRNAs act as ligands able to bind and activate a receptor in a hormone-like fashion might have broader implications that could go beyond cancer, e.g., for autoimmune diseases and inflammatory diseases. These studies reveal a mechanism by which cancer cells cross-talk with surrounding immune cells and induce them to release cytokines that increase the inflammatory response.
The present study demonstrates the importance of the tumor microenvironment in cancer growth and dissemination and provides molecular targets for the development of anticancer treatments.