Investigating the Role of the mirn23a Cluster in THP-1 Human Macrophage Polarization

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Macrophages are the primary class of immune cells employed by the body to engulf and digest other cells. They have a number of different targets, from foreign pathogens such as bacteria and parasites to cancer cells. They exist on a spectrum of activity and are able to polarize into two different forms: “classically activated” M1 macrophages that kill tumor cells and “alternatively activated” M2 macrophages that protect tumor cells and facilitate cancer progression. Tumors are able to recruit macrophages in their vicinity and release signals that polarize these cells to tumor-protecting M2 macrophages.

One way in which macrophages are able to polarize is through differential expression of microRNAs, short RNA molecules that regulate cell activity by binding to the larger RNA molecules that make proteins and targeting them for degradation. We are interested in how the mirn23a cluster—a cluster of DNA that produces three mature microRNAs—is responsible for producing different types of macrophages. We will also explore whether the manipulation of microRNA expression can skew macrophages to the M1 tumor-cytotoxic class. This manipulation would constitute a potentially novel therapeutic option in treating cancers given that it is immunologically based rather than reliant on traditional chemotherapeutics or radiation.

In this study, we infected human monocytes with a virus containing the genetic information for the mirn23a cluster, establishing an “overexpression” model in which cells infected with the virus contain increased amounts of mature microRNA genetic material. We then polarized these human monocytes to M1 and M2 macrophages, collected RNA from these cells, and analyzed the RNA to determine the degree of macrophage polarization using quantitative polymerase chain reaction (qPCR).

To differentiate between M1 and M2 macrophages, we looked at the relative fold changes in the amount of RNA coding between the “overexpression” model and the control infected with the empty virus for proteins characteristic of either M1 or M2 macrophages. We hypothesized that overexpression of the mirn23a cluster would skew macrophages to show elevated levels of RNA for M1 markers and

decreased levels of M2 markers relative to the control macrophages. As we hypothesized, we found that the overexpression of the mirn23a cluster in M1 macrophages resulted in elevated levels of RNA associated with M1 proteins. Contrary to our hypothesis, we also found that overexpression resulted in elevated levels of RNA associated with M2 proteins in M2 macrophages. Interestingly, none of these fold changes in RNA of the overexpressing macrophages relative to control macrophages were statistically significant.

The results of this study must be explored in order to understand how the overexpressed mirn23a cluster produces elevated levels of RNA coding for both M1- and M2-characteristic proteins in their respective polarization conditions. Because the microRNA cluster codes for three individual mature microRNAs, the activities of each mature microRNA may be prevalent in different cell signaling pathways.

The individualistic and cooperative roles of these mature microRNAs are likely quite complex. As a result, it is important that this study is carried out again so that these results might be validated and carry more statistical power. It may also be helpful to perform additional experiments to assess relative levels of M1 and M2-associated proteins, rather than solely focus on the RNA that makes these proteins. If the lack of statistically significant fold changes is still observed upon the repetition of this study, we may need to re-evaluate the extent of the mirn23a cluster's ability to affect macrophage polarization. Nonetheless, microRNAs and the mirn23a cluster should continue to be researched because they constitute a potential therapeutic option that could spare patients from the negative side effects of traditional anticancer therapies.