A systematic imaging-based screen to identify the microRNA regulatory landscape of the Nrf2 pathway

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Introduction

MicroRNAs are small non-coding RNAs. They regulate gene expression at the post-transcriptional level and are involved in many biological processes. Recent studies indicate that circulating microRNAs are stable and can be assessed in a non-invasive manner, making microRNAs interesting biomarker candidates. Furthermore, microRNA expression changes are linked to various diseases like cancer and neurodegenerative disorders. Moreover, research considering microRNAs showed an increasing importance of these small noncoding RNAs in different cellular stress response pathways, including e.g. oxidative stress, unfolded protein, and DNA damage response. So far, a systematic analysis of the functional role of all known microRNAs on individual stress response pathways is lacking.

Objective

Main objective is to determine the effect of microRNAs on the Nuclear factor-erythroid-2-related factor 2 (Nrf2) pathway (oxidative stress response).

Method

To monitor the Nrf2 pathway, different stress response reporter cell lines were used e.g. HepG2-Nrf2-GFP and HepG2-Srxn1-GFP, a direct downstream target of Nrf2. CDDO-Me (30 nM) was used to activate the Nrf2 pathway. MicroRNA mimics (2600) were used to enhance the effect of the endogenous microRNAs in order to detect their possible function in the Nrf2 pathway. Single cell live confocal imaging was used to measure the Nrf2-response after treatment. HepG2-Chop-GFP and HepG2-P21-GFP cells were used to investigate the effect of defined microRNA hits on the unfolded protein response and DNA damage response respectively. Whole transcriptome analysis was performed on HepG2 wild-type cells transfected with microRNAs or siRNAs to obtain information at gene level.

Results/Conclusion

We identified a set of 26 microRNAs able to alter the expression of the Nrf2 pathway. miR-1293 and miR-6499-3p were found to be the strongest inducers, while miR-200a-5p and miR-502-5p were found to be the strongest inhibitors of the Nrf2 pathway. Gene expression correlated with their protein products. Moreover, most Nrf2-enhancing microRNAs were also found to enhance Chop induction. Interestingly, the identified 26 microRNAs are known to play a role in cancer and neurodegenerative disorders like Alzheimer's disease.