Effect of miR-454 on LRIG1 Expression in Breast Cancer Cells

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ABSTRACT

Introduction: Micro RNAs (miRNAs) are regulatory RNAs that have been implicated in many human diseases. Namely, miR-454 has been demonstrated to contribute to tumorigenesis and is known to be elevated in human tumor cells. In this study, we examined the regulatory control of miR-454 on a breast cancer tumor suppressor LRIG1.

Methods: To determine the effect of miR-454 on LRIG1 expression, cells were transfected with miR-454 mimic or an antagomiR, and LRIG1 levels were subsequently assessed. A luciferase reporter assay was used to determine if miR-454 bound directly to the LRIG1 3'UTR.

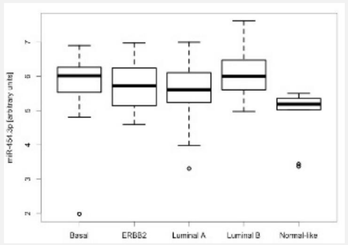
Results: LRIG1 expression levels were elevated in MCF-7 cells expressing antagomiR-454. MCF-7 and MDA-MB-231 cells expressing exogenous LRIG1 3'UTR and miR-454 had greater luciferase activity compared to cells expressing control 3'UTR and control miRNA. Exogenous expression of LRIG1 3'UTR and antagomiR-454 in MCF-7 cells, but not MDA-MB-231 cells, resulted in decreased luciferase activity relative to control cells.

Discussion: In MCF-7 cells, an ER$^+$/PR$^{+/-}$/HER2$^-$ breast cancer cell line, expression of antagomiR-454 led to increased expression of LRIG1, suggesting that miR-454 may be involved in suppressing LRIG1 expression. The results of the luciferase reporter assay were inconclusive and further studies have to be done to determine whether miR-454 regulates LRIG1 expression directly, or indirectly by modulating regulators of LRIG1.

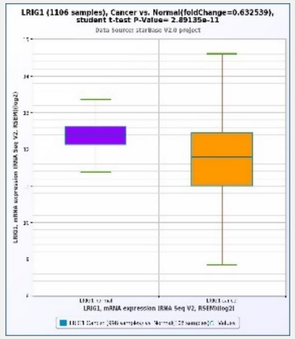
INTRODUCTION

Micro RNAs (miRNAs) are a class of regulatory RNAs in animals, plants, and viruses that play important roles in a variety of biological processes including development, cellular differentiation and proliferation, and apoptosis. To perform their regulatory function, miRNAs combine with Argonaute family proteins into miRNA-induced silencing complexes. These complexes bind to fully or partially complementary mRNA targets, typically the 3'UTR, to silence them post-transcriptionally[1]. Alternative regulatory mechanisms have been identified, including repression of transcription initiation and miRNA-mediated mRNA decay[2]. Given their vast role in gene expression regulation, hundreds of miRNAs have already been implicated in human diseases, including metabolic disorders and cancer. miRNA expression profiles have identified specific miRNAs that are overexpressed in disease states. Specifically, miR-454 was found to be upregulated in the ER$^+$/PR$^{+/-}$/HER2$^-$ MCF-7 breast cancer cell line, and was demonstrated to contribute to tumorigenesis, presumably by downregulating tumor suppressor genes[3, 4]. One such tumor suppressor whose expression is regulated by miRNA is LRIG1[5]. LRG1 is a transmembrane protein that interacts with several receptor tyrosine kinases, including those belonging to the EGFR, MET, and RET families. Recent studies have demonstrated that LRIG1 is a negative regulator of the Met receptor and it suppresses the synergy between Met and the ErbB family member, Her2[6]. LRIG1 downregulation has been observed in Her2-induced murine mammary tumors and in many Her2$^+$ human breast tumors[7]. In this study, we hypothesized that LRIG1 expression is regulated by miR-454 in several breast cancer cell lines. Our preliminary analyses of data from The Cancer Genome Atlas demonstrated that increased miR-454 levels correlated with a reduction in LRIG1 expression in human tumor cells. Other early studies also suggested that miR-454 repressed LRIG1 expression in MDA-MB-468 triple-negative breast cancer cells (results not shown). To further characterize the role of miR-454 on LRIG1 regulation in breast cancer cells, we aim to expand our analysis to various breast cancer cell lines. Further, given the high degree of sequence complementation between miR-454 and the LRIG1 3'UTR, we examined if there was a direct interaction between the two molecules that could consequently mediate LRIG1 regulation.

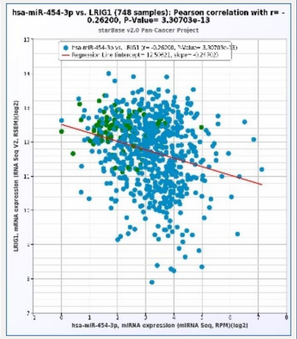
RATIONALE



MiR-454 is elevated in human tumor cells relative to normal cells.



LRIG1 mRNA levels are reduced in human tumor cells relative to normal cells (n=1106; p<0.005).



Increased miR-454 levels correlate with decreased LRIG1 mRNA expression in human tumor cells (n=748; p<0.005).

MiR-454 has high complementarity with the 3'UTR of LRIG1.

The Cancer Genome Atlas http://cancergenome.nih.gov/

METHODS

To assess endogenous LRIG1 and miR-454 levels, MCF-10A, MDA-MB-231, MCF-7, ZR75-1, BT-20, and MDA-MB-468 cells were grown to 90% confluency on 10cm tissue culture plates. Cells were lysed using a mild detergent lysis buffer, and the whole cell lysates were probed for LRIG1 via Western blotting. To assess the effect of miR-454 on LRIG1 expression, MDA-MB-231, MCF-7, and ZR75-1 cells were transfected with miR-454 mimic or scramble control miRNA +/- LRIG1 expression vector or empty control vector. Cell were lysed as previously mentioned, and whole cell lysates were probed for LRIG1 via Western blotting. To determine if there was a direct interaction between the LRIG1 3'UTR and miR-454, a luciferase reporter assay was done in which the 3'UTR of LRIG1 was inserted downstream of the luciferase gene. MCF-7, MDA-MB-231, and ZR75-1 cells were then transfected with this construct +/- miR-454 or its antagomir and luciferase activity was measured.

RESULTS

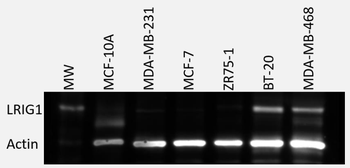


Figure 1. Enogenous LRIG1 expression in various breast cancer cell lines. Whole cell lysates from MCF-10A, MDA-MB-231, MCF-7, ZR75-1, BT-20, and MDA-MB-468 were used to measure LRIG1 expression levels via Western blotting. BT-20 and MDA-MB-468 cells demonstrated the highest levels of endogenous LRIG1 expression, while MDA-MB-231, MCF-7, and ZR75-1 cells had moderate levels.

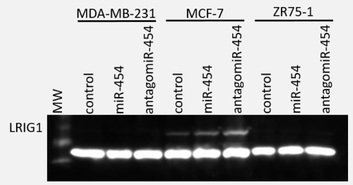


Figure 2. LRIG1 expression is higher in MCF-7 cells expressing antagomiR-454. MDA-MB-231, MCF-7, and ZR75-1 cells were transfected with LRIG1 expression vector +/- control miRNA, miR-454 mimic, or antagomiR-454. LRIG1 expression in cells transfected with miR-454 mimic was similar to expression in control cells. However, in MCF-7 cells expressing antagomiR-454, LRIG1 expression was markedly elevated suggesting that the depletion of endogenous miR-454 levels correlated with an increase in LRIG1 expression. MDA-MB-231 and ZR75-1 cells did not demonstrate any detectable changes in LRIG1 expression following any of the miRNA treatments.

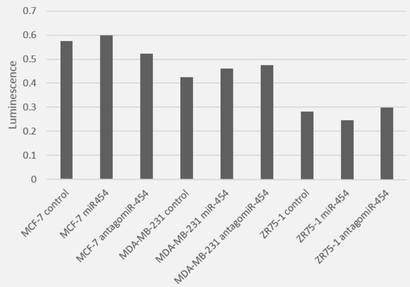


Figure 3. miR-454 interaction with the LRIG1 3'UTR. MCF-7, MDA-MB-231, and ZR75-1 cells were transfected with the LRIG1 3'UTR-luciferase gene construct +/- miR-454 or its antagomir, and luciferase activity was measured. There was an increase in luciferase activity in MCF-7 cells transfected with miR-454. In cells expressing the antagomiR, luciferase activity was reduced below control levels. Luciferase activity in MDA-MB-231 and ZR75-1 cells did not follow a similar pattern. Thus, these results are inconclusive and do not clearly demonstrate the direct interaction between miR-454 and LRIG1.

DISCUSSION

Abnormal expression of miRNA has been observed in human diseases, including breast cancer. Several miRNAs have been identified that target tumor suppressor genes to promote tumor growth and proliferation. In this study, we aimed to determine if miR-454 targeted the tumor suppressor LRIG1 in various breast cancer cells lines. We first assessed endogenous LRIG1 expression levels in MCF-10A, MDA-MB-231, MCF-7, ZR75-1, BT-20, and MDA-MB-468. Given that MDA-MB-231, MCF-7, and ZR75-1 cells exhibited moderate LRIG1 expression, we used these cell lines to assess the effect of miR-454 on LRIG1 expression. MDA-MB-231, MCF-7, and ZR75-1 cells were transfected with miR-454 mimic, antagomiR-454, or control miRNA, and evaluated for LRIG1 expression levels. MCF-7 cells transfected with antagomiR-454 showed increased levels of LRIG1 relative to control cells. miR-454 or antagomiR-454 expression did not detectably alter LRIG1 expression in MDA-MB-231 or ZR75-1 cells. This data suggests that reducing miR-454 levels can increase the expression of the tumor suppressor LRIG1 in breast cancer cells. To determine if miR-454 has a direct interaction with the 3'UTR of LRIG1 to regulate its expression, we conducted a luciferase reporter assay. MDA-MB-231, MCF-7, and ZR75-1 cells were transfected with miR-454 mimic, antagomiR-454, or control miRNA +/- LRIG1 3'UTR or control 3'UTR. The results of this experiment were inconclusive and further studies have to be done to demonstrate whether miR-454 regulates LRIG1 expression directly or indirectly by modulating other regulators of LRIG1. While this data is preliminary, taken together, it suggests that miR-454 downregulates the expression of the LRIG1 3'UTR in certain breast cancer cell lines. MDA-MB-231 is a triple-negative breast cancer cell line, while MCF-7 is an ER$^+$/PR$^{+/-}$/HER2$^-$ breast cancer cell line. In contrast to both of those cells, ZR75-1 is an ER$^+$/PR$^{+/-}$/HER2$^+$ breast cancer cell line, which could offer an explanation as to why miR-454 did not affect LRIG1 levels in these cells. LRIG1 is known to suppress the synergy between Her2 and Met. Hence, we can speculate that in HER2$^+$ cells, LRIG1 is already substantially downregulated to promote Her2-Met synergy. Further studies will have to be done to further characterize the role of miR-454 on LRIG1 expression regulation, and assess the importance of miR-454 in the progression of specific breast cancer types.

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