MicroRNA-221/222 confers lapatinib resistance through targeting p27^{kip1} expression.

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Abstract

Purpose

Blockade of HER2 signaling pathway by lapatinib, an EGFR/HER2 inhibitor, has been shown to improve the survival benefits in HER2-positive breast cancer patients. However, the clinical outcome is severely limited by the development of acquired resistance. However, the mechanisms of lapatinib resistance are not fully understood.

Materials and Methods

The change in microRNA expression in response to lapatinib treatment was identified by microRNA microarray analysis. Biochemical and molecular biological assays were used to demonstrate the involvement of downregulation of p27^{kip1} by miRNA in the development of lapatinib resistance.

Results

Elevation of miR-221 and miR-222 were found in lapatinib-resistant cells. The elevated miR-221 and miR-222 contributed to the development of acquired lapatinib resistance by targeting p27^{kip1} tumor suppressors.

Result

miR-221 and miR-222 were up-regulated in lapatinib-resistant cells.

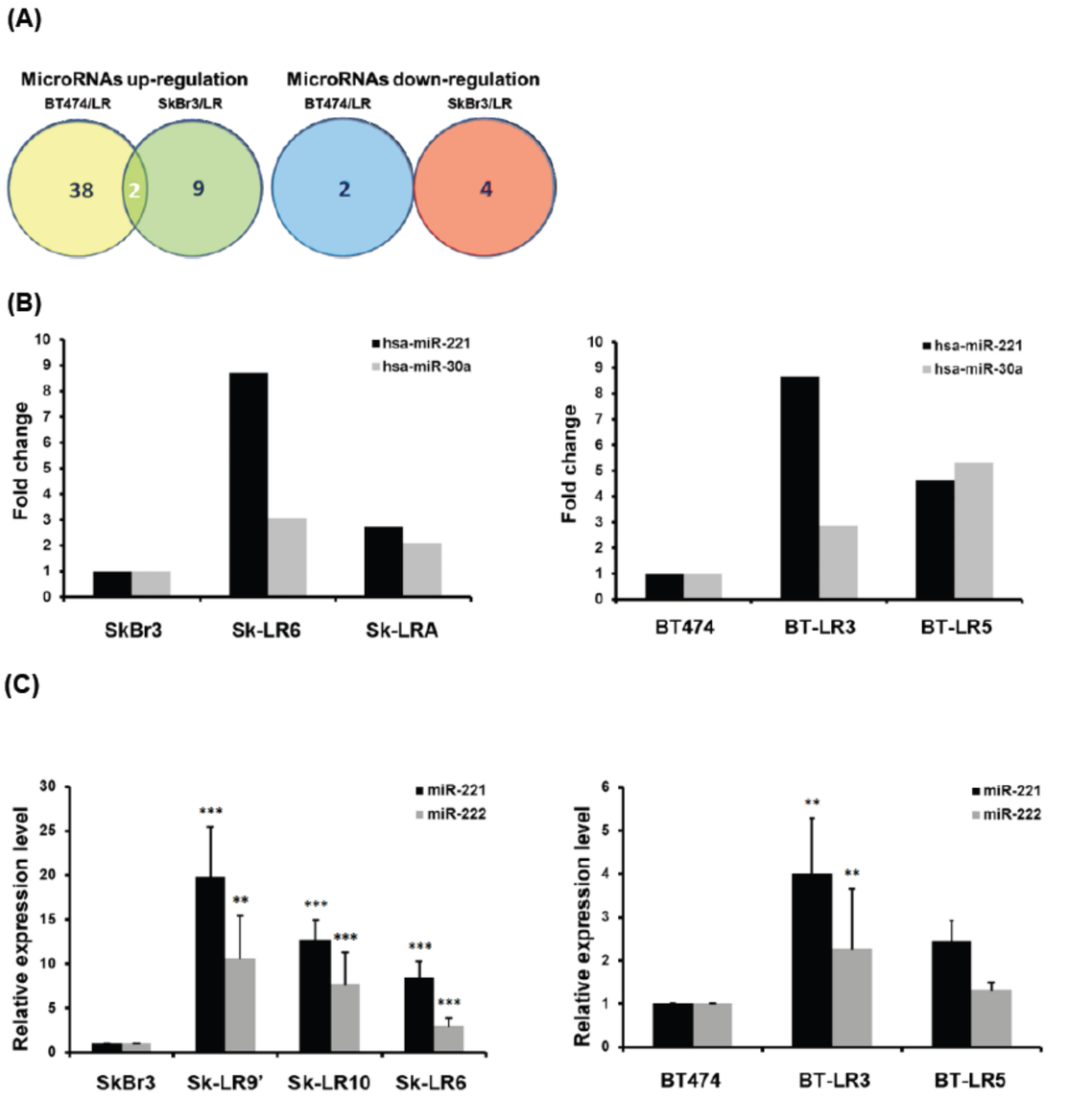

Fig 1. miR-221 and miR-222 expression levels in Lap^r breast cancer cell lines. (A) MicroRNA array analysis for expression profiling in lapatinib-treated breast cancer cells and Lap^r breast cancer clones. (B) Quantitative of miRNA microarray. (C)Total RNA containing miRNA was extracted from Lap^r breast cancer cells (SkBr3/LR6 and BT474/LR3) and subjected to quantitative RT-PCR for miR-221 and miR-222. Data are expressed as mean ± SD (n=3).

Elevation of miR-221 and miR-222 contributes to lapatinib resistance.

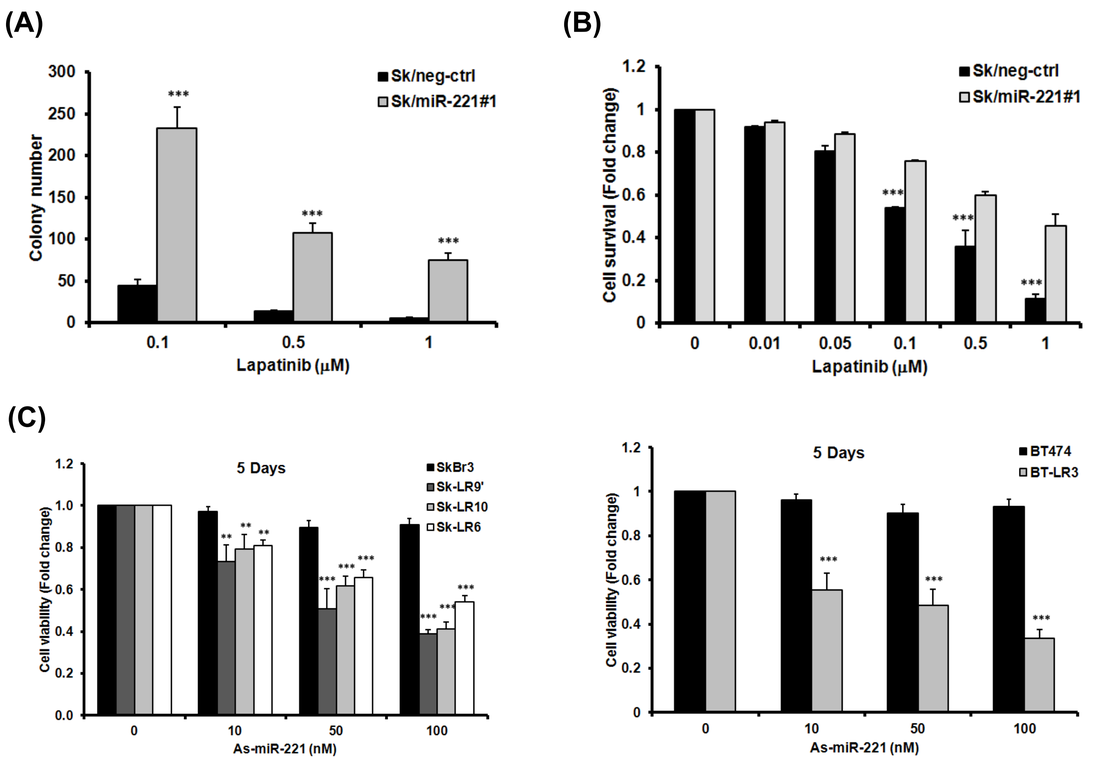

Fig. 2. miR-221 play a role in lapatinib-resistance breast cancer cell. (A) Overexpression of miR-221 caused lapatinib-resistance in breast cancer cell lines by colony assay. (B) Overexpression of miR-221 caused lapatinib-resistance in SkBr3 in MTT assay. (C) Silencing of miR-221 decreased cell viability in SKBR3, SK/LR clones (left), BT474, and BT/LR3 (right). Data are expressed as mean ± SD (n=3).

Lapatinib-induced miR-221 and miR-222 negatively regulated p27^{kip1} expression to suppress apoptosis

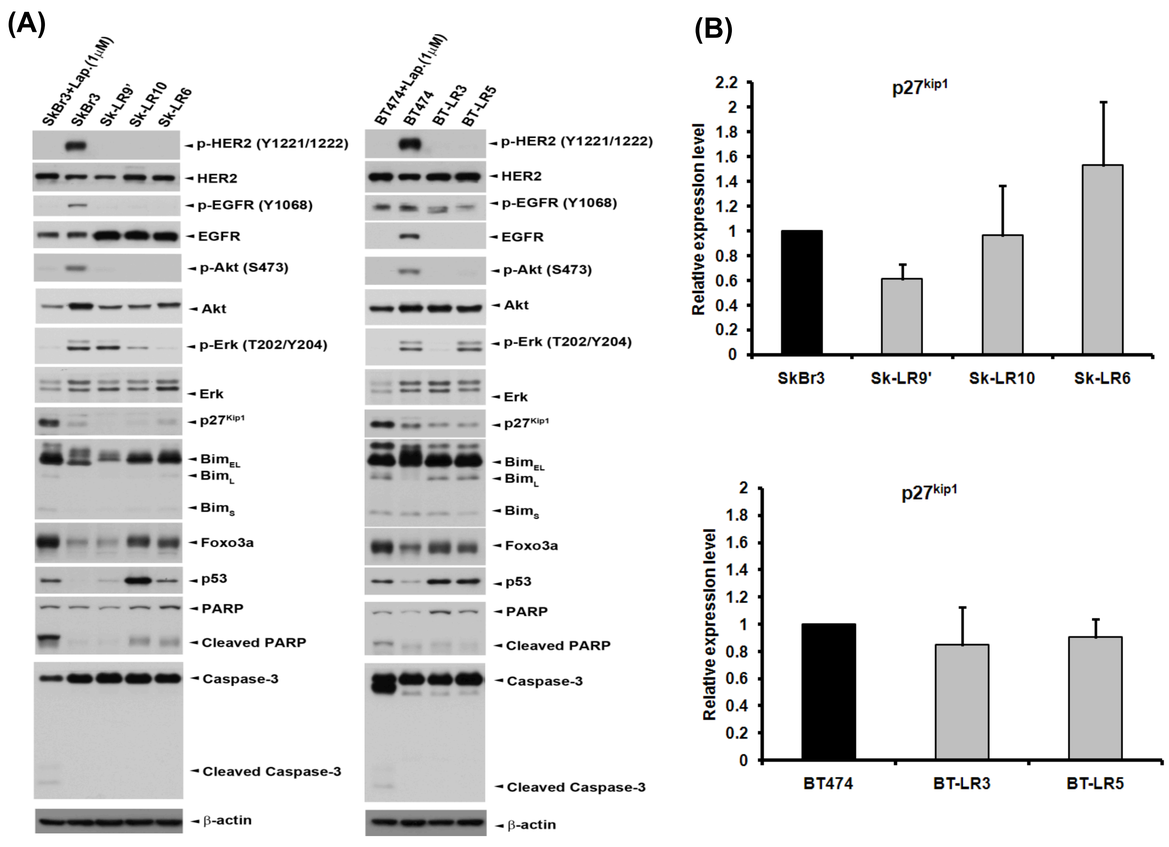

Fig. 3. p27kip1 Is Negatively Regulated by miR-221. (A) Immunoblot analysis for determinate the protein changed in SKBR3, SK/LR clones (left), BT474, and BT/LR clones (right). (B) Quantitative of p27^{kip} mRNA level in SKBR3, SK/LR clones (top), BT474, and BT/LR clones (bottom). (C) overexpression of miR-221 in SKBR3 (left) and BT474 (right) parental cells. (D) Quantitative analysis for p27 mRNA level (left) and immunoblot analysis for the protein expression (right)

The Regulation of p27^{kip1} by miR-221.

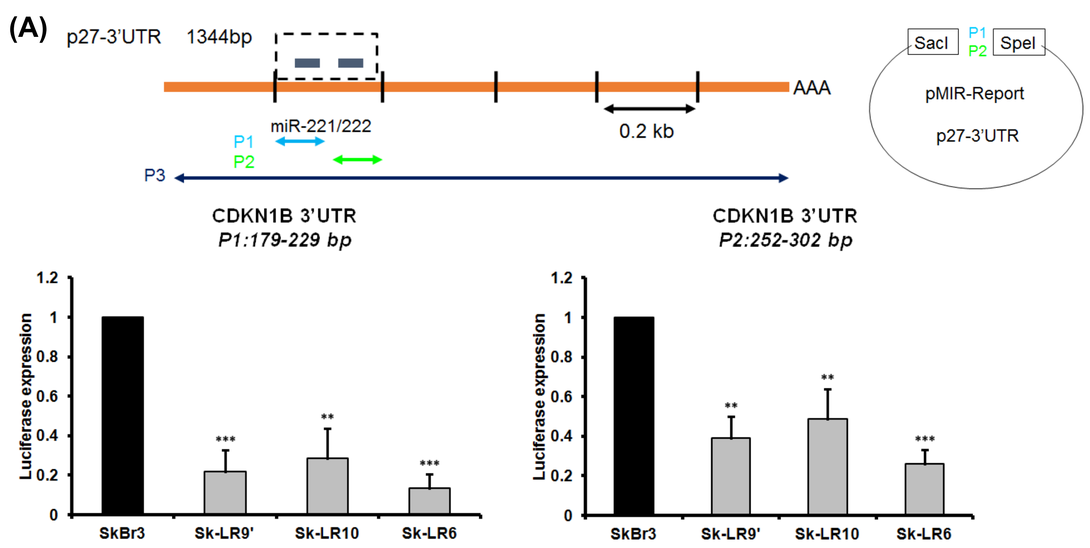

Fig. 4. The Regulation of p27^{kip1} by miR-221. (A) Transfected with CDKN1B 3'-UTR constructs into SKBR3 and SK/LR clones. (B) Transfected with CDKN1B 3'-UTR constructs into SKBR3 and SK/miR221 clones. (C) current model.

Conclusion

Our results demonstrated the critical role of miR-221 and miR-222 in conferring lapatinib resistance through directly targeting p27^{kip1}- 3'UTR to suppress p27^{kip1} protein expression.