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Loss of miR-155 upregulates WEE1 in metastatic melanoma

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Abstract

Significant advances have been made in the treatment of melanoma by targeting key cellular pathways, but additional targets are needed as many patients do not respond or relapse with resistant disease. MicroRNA-155 (MiR-155) has previously been shown to regulate melanoma cell growth and acts as a tumor suppressor. We tested a clinical population of melanoma tumors for miR-155 expression, and find that expression is low in most patients, although not predictive of outcome. We identified the protein kinase WEE1 as a novel target of miR-155. A mouse model of experimental metastasis finds that both increased expression of miR-155 and silencing of WEE1 lead to decreased metastases. Loss of miR-155 and increased expression of WEE1 may contribute to the metastatic phenotype in patients with melanoma.

Keywords

melanoma; metastasis; microRNA; microRNA-155; WEE1

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Conflicts of interest

There are no conflicts of interest.

Introduction

The incidence of melanoma continues to increase faster than any other cancer in the USA [1]. Recent advances in targeted and immunotherapy have led to real survival gains for a minority of patients who either present with or develop metastatic disease. There are several known molecular pathways involved in melanoma development that offer potential treatment targets. The most significant known mutations are in *BRAF* (which occurs in ≤ 66% of melanomas), *NRAS* (in 25–35%) and *CDKN2A* (in 10%) [2]. Despite success with these recent therapies, many patients do not respond or the response is not durable [3]. Recent interest has focused on microRNAs as prognostic indicators, and they may have potential as treatment targets in melanoma.

MicroRNAs are small, noncoding RNA that are involved in post-transcriptional regulation of gene expression and have been implicated in oncogenesis [4–6]. MicroRNAs are processed from pre-miRs in the cytoplasm into mature microRNAs, which are single-stranded and ~ 22 nucleotides. Each microRNA has a seed region from nucleotides 2 to 8 with complementarity to the mRNAs they regulate. miRNA binding results in degradation of target mRNA and/or inhibition of its translation [7].

MicroRNA-155 (miR-155) was first discovered to play a role in B-cell lymphoma [8]. It is located on chromosome 21, in exon 3 of BIC/MIR155HG [9]. It has also been found to play a role in papillary thyroid cancer, pancreatic ductal adenocarcinoma, breast cancer and lung cancer [10]. MiR-155 is most commonly found to be upregulated in malignancy and is hypothesized to target tumor suppressor genes such as SOCS1 and APC [11].

Melanoma cell lines have been shown to have very low levels of miR-155, although the downstream effect of this loss of expression is unknown [12,13]. Levati *et al.* [12] found that reintroduction of miR-155 was able to inhibit proliferation and induce apoptosis in melanoma cell lines. They further found that miR-155 controls the expression of SKI, a transcriptional co-regulator that is over-expressed in melanoma cell lines compared to normal melanocytes. SKI was not found to be a critical mechanism in growth suppression with miR-155, however, suggesting that miR-155 controls other pathways as well [14]. Segura *et al.* [15] found that in patient tumor samples there was an increase in miR-155 in patients with longer survival compared to those with shorter survival, suggesting that there is a loss of miR-155 with the development of a more malignant phenotype. MiR-155 seems to play a role in melanoma tumor suppression, but the mechanism is unclear.

WEE1 is a protein kinase in the BRAF pathway and is involved in regulating the cell cycle and tumor progression in several cancer types [16]. WEE1 negatively regulates entry into mitosis by phosphorylating cyclin-dependent kinase 1 (CDK1), inactivating the CDK1/cyclin B complex and arresting the cell cycle at the G2/M checkpoint. We have previously shown in melanoma that WEE1 is over-expressed in patient tumor samples compared to normal melanocytes, as well as in melanoma cell lines. Additionally, experimentally induced down-regulation of WEE1 was shown to inhibit melanoma cell growth in cell culture and in animal disease models [17].

Results

In this study, a group of melanoma patient tumor samples were examined for miR-155 expression levels and correlated with clinical outcome. Melanoma cell lines and xenograft tumor development were used to explore a possible mechanism by which miR-155 modulates WEE1 expression.

MiRNA microarray analysis (Affymetrix, Thermo Fisher Scientific, Waltham, Massachusetts, USA) was performed on melanoma specimens from patients, with confirmation by qRT-PCR. Prior studies have identified miR-155 as one of several miRNAs that predict outcome in select groups of patients, such as stage III [15,18]. In our patient tumor samples, miR-155 levels were low, but miR-155 could not be correlated with development of metastasis or overall patient survival (Supplementary Fig., Supplemental digital content 1, http://links.lww.com/MR/A81). Other miRNAs were more strongly associated with the development of metastatic disease, although none independently predicted survival. These miRNAs are being separately studied (Supplementary Fig., Supplemental digital content 1, http://links.lww.com/MR/A81).

We next sought the target of miR-155, looking for homology in the 3'-UTR, and used MiRanda (http://www.microrna.org/ [19]), and TargetScan (http://www.targetscan.org/ [20]). WEE1 was predicted as a potential target with complementarity to the seed sequence (Fig. 1a). We confirmed the interaction between miR-155 and WEE1 using a luciferase reporter assay. The 3′-UTR was cloned into a plasmid containing the firefly luciferase reporter gene. The seed sequence to miR-155 was deleted from the 3′-UTR using the QuikChange II site-directed mutagenesis kit (Agilent Technologies, Santa Clara, California, USA). A metastatic melanoma cell line (UACC 903M) was transfected with either the luciferase plasmid with the intact WEE1 3′-UTR or with the seed sequence deleted (WEE1del) and with either microRNA-NC negative control, miR-155 mimic or miR-155 inhibitor. When miR-155 was present there was a significant decrease in luciferase activity ($P = 0.003$; Fig. 1b). When the seed sequence for miR-155 is deleted in the 3′-UTR of WEE1 the effect of miR-155 mimic on the luciferase activity is lost.

The control of miR-155 on WEE1 was further tested by manipulating miR-155 levels and assessing WEE1 protein levels. Metastatic melanoma cell lines UACC 903M and A375M have low levels of miR-155. These cell lines were transfected with miR-155 mimic, which reduced WEE1 protein levels, while inhibition of endogenous miR-155 up-regulated WEE1 expression at both 48 and 96 h post-nucleofection (Fig. 1c).

WEE1 has previously been shown to arrest the melanoma cell cycle, leading to cell death [17]. We confirmed this in our melanoma cell lines, and further show that miR-155 has a similar effect. WEE1 expression was silenced in three melanoma cell lines (UACC 903M, A375M, and 1205Lu) by siRNA, which results in a significant decrease in cell viability in an MTS assay (Fig. 2a, 1205Lu, UACC 903M, A375M). Decreased cell viability is also seen with the addition of miR-155 mimic, although less than with siWEE1.

The in-vitro findings were confirmed in a mouse model of experimental metastasis. We chose the 1205Lu cell line, as we saw the most robust suppression in the MTS assay.

The 1205Lu cells were nucleofected (Amaxa, Lonza, Walkersville, Maryland, USA) with miR-155 mimic and injected into the tail veins of nude mice. The miR-155 transfected cells produced significantly fewer lung metastases than the control cells (Fig. 2b). A similar experimental metastasis model was performed using siRNA for WEE1. Similar to miR-155 restoration, when WEE1 is silenced the number of metastases decreases (Fig. 2c and d).

WEE1 is a protein kinase involved in regulation of the cell cycle. WEE1 phosphorylates and inactivates CDK1 and acts as a part of the G2/M checkpoint [21]. Previous studies have shown that WEE1 is overexpressed in melanoma cells compared to normal melanocytes [17]. WEE1 was found to be a key signaling molecule downstream of ^{V600E}BRAF in the MAPK signaling cascade [17]. The mechanisms leading to over-expression of WEE1 are not previously described. WEE1 is a predicted target of miR-155, which has been previously reported to be lost in melanoma cells [12]. In this study, we show that WEE1 is a target for miR-155. Treating metastatic melanoma cells with miR-155 mimic or silencing WEE1 results in decreased cell viability *in vitro* and decreased growth of metastases in our in-vivo studies.

Conclusion

Results from this study suggest that miR-155 expression is lost in patients who develop metastatic melanoma. MiR-155 regulates the expression of WEE1, which in turn regulates the cell cycle. Both overexpression of miR-155 and silencing of WEE1 result in a significant decrease in metastasis in a mouse model. This suggests that in melanoma miR-155 controls metastasis through the WEE1 kinase. Further research should elucidate the role of miR-155 and WEE1 in relation to other cellular processes that drive melanoma growth and metastasis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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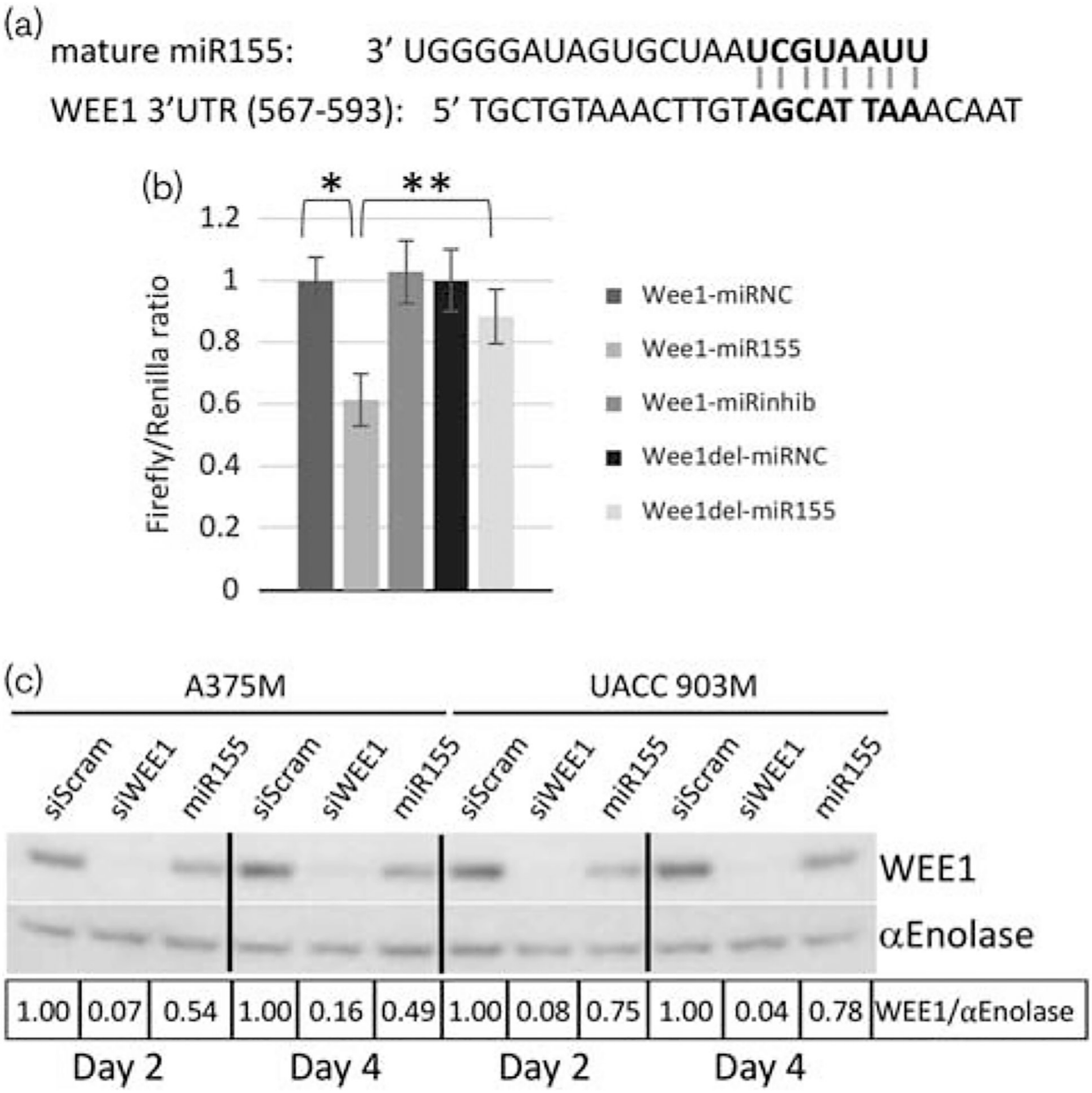

Fig. 1.

(a) Seed sequence for microRNA-155 (miR-155) and 3′-UTR of WEE1. (b) Luciferase reporter assay using plasmid with 3′-UTR of WEE1 and plasmid with 3′-UTR of WEE1 with miR-155 seed sequence deleted. *P = 0.003, **P = 0.04. (c) Expression of WEE1 kinase in A375M and UACC 903M melanoma cell lines nucleofected with either siScrambled, siWEE1, or miR-155 mimic at 48 and 96 h post-nucleofection.

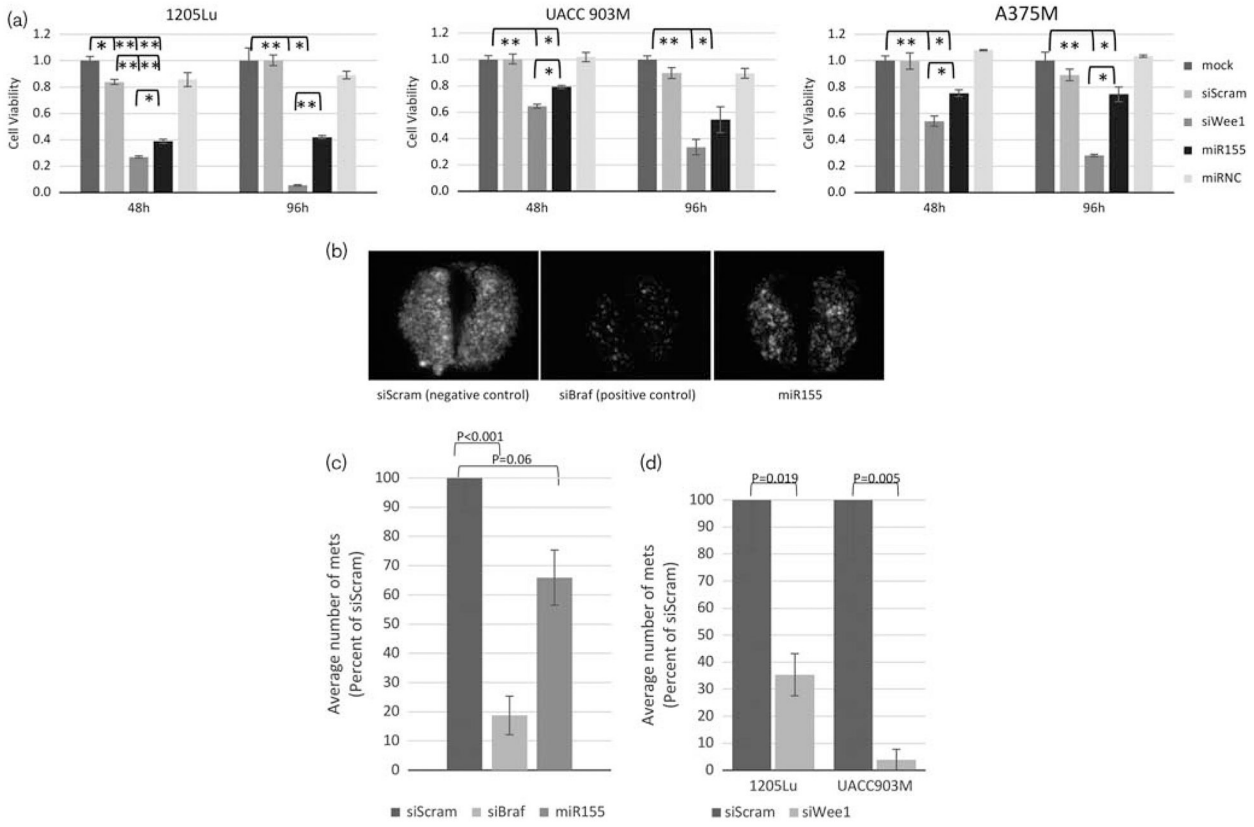

Fig. 2.

(a) Cell viability (MTS assay): siRNA targeting WEE1 and microRNA-155 (miR-155) mimic reduced cell viability in multiple cell lines. siScrambled and miR-NC were used as negative controls. Data representative of a single experiment done in triplicate and are represented as means, SEM, percentage of cell viability.*$P < 0.05$,**$P < 0.01$. (b) Representative images of mouse experimental metastasis model showing lungs of mice 21 days after tail vein injection of 1205Lu cells nucleofected with either siScrambled (negative control), siBraf (positive control) or miR-155 mimic RNA. (c) Mouse experimental metastasis using 1205Lu with miR-155 mimic. (d) Mouse experimental metastasis using 1205Lu with siWEE1.