Biochemical and Biophysical Research Communications 430 (2013) 706–710

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

miRNA-7-5p inhibits melanoma cell migration and invasion

Keith M. Giles [a,1], Rikki A.M. Brown [a,1], Michael R. Epis [a], Felicity C. Kalinowski [a], Peter J. Leedman [a,b,*]

[a] Laboratory for Cancer Medicine, Western Australian Institute for Medical Research and University of Western Australia Centre for Medical Research, Perth, WA 6000, Australia
[b] School of Medicine and Pharmacology, University of Western Australia, Nedlands, WA 6008, Australia

ARTICLE INFO

Article history:
Received 14 November 2012
Available online 1 December 2012

Keywords:
microRNA
Melanoma
Migration
Invasion
Metastasis
Signaling

ABSTRACT

Aberrant expression of microRNAs (miRNAs), a class of small non-coding regulatory RNAs, has been implicated in the development and progression of melanoma. However, the precise mechanistic role of many of these miRNAs remains unclear. We have investigated the functional role of miR-7-5p in melanoma, and demonstrate that miR-7-5p expression is reduced in metastatic melanoma-derived cell lines compared with primary melanoma cells, and that when ectopically expressed miR-7-5p significantly inhibits melanoma cell migration and invasion. Additionally, we report that insulin receptor substrate-2 (IRS-2) is a target of miR-7-5p in melanoma cells, and using RNA interference (RNAi) we provide evidence that IRS-2 activates protein kinase B (Akt), and promotes melanoma cell migration. Thus, miR-7-5p may represent a novel tumor suppressor miRNA in melanoma, acting at least in part via its inhibition of IRS-2 expression and oncogenic Akt signaling.

1. Introduction

Melanoma is an aggressive form of skin cancer that is increasing in incidence globally, especially in fair skinned individuals. When metastatic, it is associated with a very poor prognosis and highly refractory to chemotherapy and radiotherapy [1]. Recent advances in understanding the molecular biology of melanoma have led to the development of new targeted therapies such as vemurafenib, which targets a BRAF V600 activating mutation and has produced significant anti-tumor responses in clinical studies [2]. Despite these impressive results, resistance to targeted therapies is common and remains a significant therapeutic challenge [3]. Thus further investigation of the molecular mechanisms underlying melanoma development and progression is urgently required to design new therapeutic strategies and improve clinical outcomes.

MicroRNAs (miRNAs) are short, endogenous, non-coding RNAs which target the 3′-untranslated region (3′-UTR) of specific mRNAs to promote their degradation or repression of translation [4]. miRNAs regulate important cellular processes such as proliferation, apoptosis, cell cycle progression, and differentiation, and their altered expression is associated with various cancers, including melanoma [5]. In addition, specific miRNAs – termed metastamiRs [6] – have been reported to regulate melanoma cell migration, invasion and metastasis [7], suggesting that they represent novel targets to inhibit melanoma progression. For example miR-34b/c act as suppressors of metastasis as ectopic expression of these miRNAs has been shown to directly target the proto-oncogene MET, thereby inhibiting MET-induced signal transduction and execution of the invasive growth program in melanoma cells [8]. Accordingly, miRNAs have significant diagnostic, prognostic and therapeutic potential, and the therapeutic delivery of miRNA inhibitors and mimics has emerged as a viable option for the treatment of cancer [9].

In this study, we investigated the functional role of microRNA-7 (miR-7-5p) in melanoma. In addition to being downregulated in a variety of cancers, miR-7-5p has been reported to decrease tumor cell proliferation, anchorage-independent growth, tumorigenicity, migration and invasion, and to promote apoptosis and chemosensitivity by repressing expression of a variety of specific oncogenic target molecules [10–15]. Of particular interest, miR-7-5p is downregulated in a metastatic versus a primary melanoma cell line established from the same patient [16], and is also downregulated in highly invasive melanoma cells compared to a less invasive derivative [17], suggesting its dysregulation may be an important feature of melanoma progression. In this study, we present the first evidence that miR-7-5p inhibits the migration and invasion of melanoma cell lines *in vitro*.

Abbreviations: miRNA, microRNA; IRS-2, insulin receptor substrate-2; IRS-1, insulin receptor substrate-1; PI3K, phosphatidylinositol 3-kinase; Akt, protein kinase B; RT-qPCR, reverse transcription and quantitative polymerase chain reaction; BRAF, v-Raf murine sarcoma viral oncogene homolog B1; RAF1, v-raf-1 murine leukemia viral oncogene homolog 1; EGFR, epidermal growth factor receptor; PAK1, p21-activated kinase 1; FAK, focal adhesion kinase; RTCA-DP, real-time cell analyzer dual-plate; CEB, cytoplasmic extraction buffer; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

* Corresponding author. Address: Western Australian Institute for Medical Research, Level 6, MRF Building, Rear 50 Murray Street, Perth, WA 6000, Australia. Fax: +618 9224 0322.
E-mail address: peter.leedman@waimr.uwa.edu.au (P.J. Leedman).
[1] Co-first authors.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.11.086

2. Material and methods

2.1. Cell culture and transfection

WM266-4 and A375 metastatic melanoma cell lines were purchased from the American Type Culture Collection (ATCC). A2058 cells were a gift from Prof. Peter Klinken (Western Australian Institute for Medical Research). The cell line A375 was derived from a primary melanoma [18], and the WM266-4 and A2058 cell lines were both derived from melanoma lymph node metastases [19,20]. Cells were cultured at 37 °C in 5% CO_2 with RPMI 1640 media (Invitrogen) supplemented with 10% fetal bovine serum (FBS). Synthetic miRNA precursor molecules (Ambion) corresponding to hsa-miR-7-5p (Product ID: PM10047) and a negative control miRNA (miR-NC; Product ID: AM171100) and Silencer Select siRNAs (Invitrogen) Negative Control No. 1 (si-NC; 4390843), si-IRS-2 #1 (s16486), si-IRS-2 #2 (s16487) were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. Cells were harvested for RNA (24 h) or protein (48 h) post-transfection for RT-qPCR and Western blot analysis.

2.2. Reverse transcription and quantitative polymerase chain reaction (RT-qPCR) and statistical analysis of data

Total RNA was isolated from WM266-4, A2058 and A375 cells using QIAzol reagent (Qiagen) as per manufacturer's instructions. For RT-qPCR analysis of miR-7-5p expression, reverse transcription and qPCR were carried out using the TaqMan miRNA assay kit (Applied Biosystems) for hsa-miR-7-5p (Part #4373014) and U44 snRNA (Part #4427975), according to manufacturer's instructions, and a Rotor-Gene 6000 thermocycler (Qiagen). For RT-qPCR analysis of IRS-2 and GAPDH mRNA expression, 0.5 μg of total RNA was reverse transcribed into cDNA using a QuantiTect Reverse Transcription Kit (Qiagen) and qPCR performed with a Rotor-Gene 6000 thermocycler (Qiagen) using a SensiMixPlus SYBR Kit (Bioline, Quantace) and IRS-2 and GAPDH primers from PrimerBank [21]: IRS2-F, 5′-CGG TGA GTT CTA CGG GTA CAT-3′; IRS2-R, 5′-TCA GGG TGT ATT CAT CCA GCG-3′; GAPDH-F, 5′-ATG GGG AAG GTG AAG GTC G-3′; GAPDH-R, 5′-GGG GTC ATT GAT GGC AAC ATT A-3′. Expression of IRS-2 mRNA (relative to GAPDH) and miR-7-5p (relative to U44 snRNA) was determined using the $2^{-\Delta\Delta Ct}$ method [22], and statistical analysis of data was performed using GENEX software (MultiD), where $p < 0.05$ in a Student's t test represented a significant difference between means.

2.3. Western blot analysis

Protein lysates were prepared from WM266-4 and A2058 cells with cytoplasmic extraction buffer (CEB) as described previously [23]. Protein samples (15 μg) were resolved on NUPAGE NOVEX 4–12% Bis-Tris gels (Invitrogen) and transferred to Immobilon-FL membranes (Millenium Science). Western blot analysis was performed using the Odyssey Western Blotting Protocol (Li-Cor), as per manufacturer's instructions. Membranes were probed with anti-IRS-2 (#4502, Cell Signaling Technology), anti-P-IRS-2 (Ser731, #ab3690, Abcam), anti-Akt (#9272, Cell Signaling Technology), anti-P-Akt (Ser473, #4060, Cell Signaling Technology), anti-ERK-1/2 (#4696, Cell Signaling Technology), anti-P-ERK-1/2 (#9101, Cell Signaling Technology) and anti-β-actin (#ab6272, Abcam) primary antibodies and fluorescently-labeled anti-mouse and anti-rabbit IRDye 680/800 (Millenium Science) secondary antibodies. Protein bands were detected and quantified with the Odyssey infrared imaging system (Li-Cor).

2.4. Migration and invasion assays

Cell migration and invasion was monitored with a Real-Time Cell Analyzer dual-plate (RTCA-DP) xCELLigence system (Roche). This instrument measures changes in the impedance of microelectric sensors (cell index) that occur as cells migrate or invade through an artificial membrane via chemotaxis [24]. For migration and invasion experiments, transfections were performed as described above. After 48 h equal numbers of cells were seeded into the upper chamber of CIM-16 plates (Roche) in serum free medium. Medium containing 20% FBS was used as a chemoattractant in the lower chambers and cell index measurements performed with the RTCA-DP device over a 25 h period. For invasion assays, the CIM-plates were coated with Matrigel (BD Biosciences; diluted at a ratio of 1:20 in serum free medium) approximately 4 h prior to seeding cells. Data represents changes in cell index over time.

3. Results and discussion

3.1. miR-7-5p expression is reduced in metastatic melanoma cell lines

miR-7-5p expression is reported to be decreased in invasive melanoma cells compared to a less invasive derivative [17], and in metastatic versus primary melanoma cell lines from the same individual [16]. TaqMan miRNA RT-qPCR assays were used to assess the expression of miR-7-5p in two cell lines derived from metastatic melanoma, WM266-4 and A2058, relative to a primary melanoma-derived cell line, A375 (Fig. 1A). A significant downregulation of miR-7-5p was observed in the metastatic melanoma lines suggesting they represent a useful model to study the mechanistic role of miR-7-5p in melanoma migration and invasion.

3.2. miR-7-5p inhibits migration and invasion of melanoma cell lines

A number of recent studies have demonstrated that miR-7-5p inhibits cell migration and metastasis in breast cancer [10,25], glioblastoma [13], gastric cancer [26] and hepatocellular carcinoma [27]. To investigate a possible functional role for miR-7-5p in melanoma cell migration and invasion, miR-7-5p expression was increased in A2058, WM266-4 and A375 melanoma cell lines following transfection with synthetic miR-7-5p mimic, and an xCELLigence system used to monitor cell migration and invasion in real-time. In each melanoma cell line, miR-7-5p overexpression significantly reduced the rate of cell migration (Fig. 1B) and invasion (Fig. 1C) over a 20–25 h period compared with a non-targeting, negative control miRNA (miR-NC). TaqMan miRNA RT-qPCR confirmed significant miR-7-5p overexpression in each melanoma cell line following transient transfection with miR-7-5p (data not shown). Together, these results indicate that miR-7-5p inhibits the migration and invasion of melanoma cell lines *in vitro*.

3.3. Insulin receptor substrate-2 (IRS-2) is a target of miR-7-5p in melanoma cell lines

miR-7-5p is a reported tumor suppressor miRNA in different cancer systems, and a number of direct, oncogenic miR-7-5p target molecules have been identified, including epidermal growth factor receptor (EGFR) [28,11], p21-activated kinase 1 (PAK1) [10,12,29], insulin receptor substrate-1 (IRS-1) [10], insulin receptor substrate-2 (IRS-2) [28,12], focal adhesion kinase (FAK) [13], and v-raf-1 murine leukemia viral oncogene homolog 1 (RAF1) [28,10,12]. We focused on IRS-2 as a candidate miR-7-5p target molecule in melanoma as it is overexpressed in a variety of metastatic

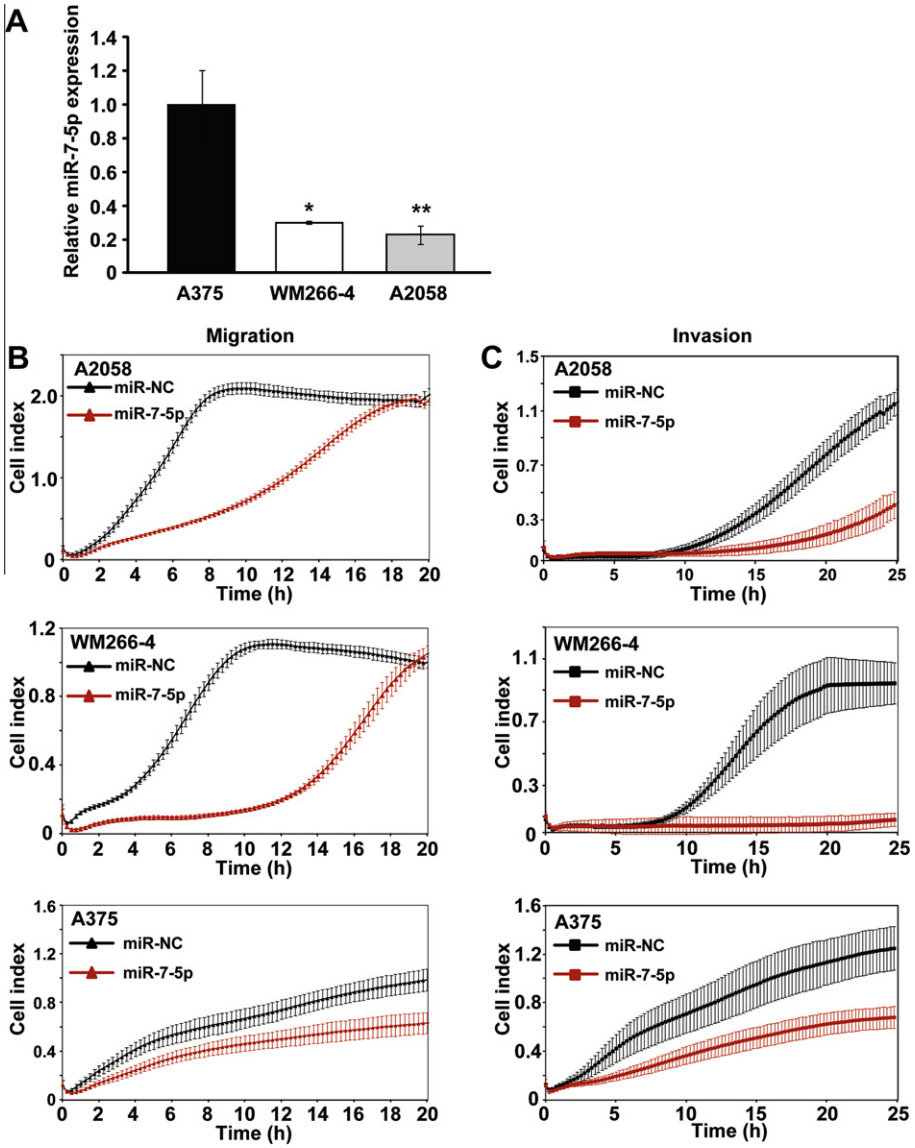

Fig. 1. miR-7-5p is downregulated in metastatic melanoma cell lines versus a primary melanoma cell line, and inhibits the migration and invasion of multiple melanoma cell lines *in vitro*. (A) TaqMan miRNA RT-qPCR analysis of miR-7-5p expression levels between A375 (primary melanoma), WM266-4 and A2058 (both metastatic melanoma) cell lines. Data is normalized to U44 snRNA expression and expressed relative to A375 cells. Error bars represent standard deviations. $^{*}p < 0.02$; $^{**}p < 0.01$. (B) Real-time xCELLigence analysis of migration (represented by cell index) of A2058, WM266-4 and A375 melanoma cell lines following transfection with miR-7-5p (red) or miR-NC (black). (C) Real-time xCELLigence analysis of invasion (represented by cell index) of A2058, WM266-4 and A375 melanoma cell lines following transfection with miR-7-5p (red) or miR-NC (black). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

cancer cell types, including uveal melanoma [30], mediates cancer cell migration [31], and activates oncogenic phosphatidylinositol 3-kinase/protein kinase B (PI3 K/Akt) signaling via direct activation of PI3K [32]. To determine whether miR-7-5p can regulate IRS-2 expression in metastatic melanoma cells, WM266-4 and A2058 melanoma cell lines were transfected with miR-7-5p and IRS-2 protein and mRNA expression were assessed by immunoblotting and RT-qPCR. For each melanoma cell line, miR-7-5p reduced expression of IRS-2 protein, as well as the active, phosphorylated form of IRS-2 (P-IRS-2) (Fig. 2A), and significantly decreased the levels of IRS-2 mRNA (Fig. 2B), suggesting that miR-7-5p promotes the degradation of IRS-2 mRNA in melanoma cell lines. Our data provide further evidence linking miR-7-5p with IRS-2 expression and support other recent reports showing miR-7-5p directly regulates IRS-2 expression in glioblastoma [11], tongue squamous cell carcinoma [12], and schwannoma cells [29].

3.4. IRS-2 inhibition decreases Akt signaling and melanoma cell migration

We next hypothesized that the capacity of miR-7-5p to inhibit melanoma cell migration and invasion *in vitro* is in part due to its regulation of IRS-2 expression. Loss of miR-7-5p expression in melanoma cells could facilitate upregulation of IRS-2 and other pro-migratory/pro-invasive miR-7-5p target molecules, thus promoting a metastatic phenotype. In this context, miR-7-5p would act as a tumor suppressor by 'fine-tuning' oncogene expression [33]. To test this hypothesis, we first performed RNAi experiments to reduce endogenous IRS-2 expression in WM266-4 metastatic melanoma cells. Transfection with two validated siRNAs against IRS-2 (si-IRS-2 #1, si-IRS-2 #2) resulted in a significant reduction in both IRS-2 mRNA and protein expression (Fig. 2C and D), as well as the levels of P-IRS-2, when compared with vehicle only (LF2000)

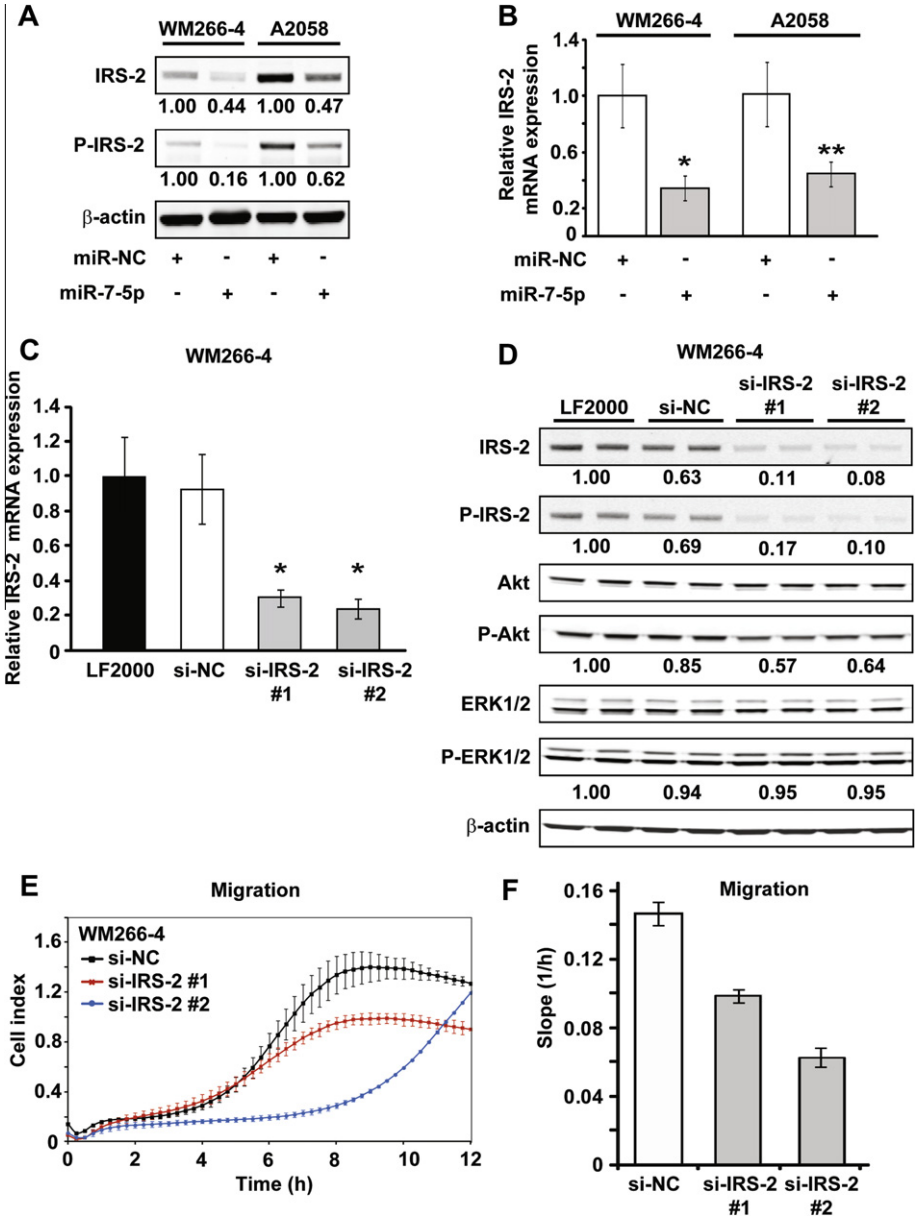

Fig. 2. IRS-2 is a target of miR-7-5p in melanoma cell lines, and IRS-2 RNAi inhibits Akt activity and melanoma cell line migration. (A) Western blot of IRS-2, P-IRS-2 and β-actin expression (loading control) 72 h after transfection of WM266-4 and A2058 cells with miR-7-5p or miR-NC. Densitometry was used to quantitate band intensity (values shown beneath each band). (B) RT-qPCR of IRS-2 mRNA expression 24 h after transfection of WM266-4 and A2058 cells with miR-7-5p or miR-NC. Data was normalized to GAPDH expression and expressed relative to miR-NC for each cell line. Error bars represent standard deviations. $^{*}p < 0.001$; $^{**}p < 0.01$. (C) RT-qPCR of IRS-2 mRNA expression 24 h after transfection of WM266-4 cells with IRS-2 siRNAs (si-IRS-2 #1, si-IRS-2 #2) or NC siRNA (si-NC). Data was normalized to GAPDH expression and expressed relative to si-NC. Error bars represent standard deviations. $^{*}p < 0.0002$. (D) Western blot of IRS-2, P-IRS-2, Akt, P-Akt, ERK1/2, P-ERK1/2 and β-actin (loading control) expression 72 h after transfection of WM266-4 cells with IRS-2 siRNAs (si-IRS-2 #1, si-IRS-2 #2) or NC siRNA (si-NC). Duplicate bands are shown and densitometry was used to quantitate band intensity (mean intensity values shown beneath each set of duplicate bands). (E) Real-time xCELLigence analysis of migration (represented by cell index) of WM266-4 melanoma cells following transfection with si-NC (black), si-IRS-2 #1 (red), or si-IRS-2 #2 (blue). (F) Graphical representation of the rate of migration (slope, 1/h) over 12 h for WM266-4 cells from (E), which were transfected with si-NC, si-IRS-2 #1, or si-IRS-2 #2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

or non-targeting siRNA (si-NC). Interestingly, the RNAi-mediated IRS-2 knockdown was accompanied with reduced activity of Akt (P-Akt; Fig. 2D), a key effector molecule downstream of IRS-2 and integral to the PI3K pathway, which regulates multiple oncogenic processes, including cell migration [34]. This effect is consistent with the established role of IRS-2 in regulating PI3K activity [32]. In contrast, there was no significant effect of IRS-2 RNAi on activity of ERK1/2 (P-ERK1/2; Fig. 2D).

To investigate the role of IRS-2 in regulating the migration and invasion of metastatic melanoma cells, we performed IRS-2 RNAi in WM266-4 cells and assessed their migration and invasion in real-time with an xCELLigence instrument. The migration rate of these metastatic cells was reduced following transfection of either si-IRS-2 #1 or si-IRS-2 #2, relative to si-NC (Fig. 2E and F). In contrast, IRS-2 RNAi did not significantly inhibit invasion of WM266-4 cells *in vitro* (data not shown). Taken together, these data are the first to demonstrate that miR-7-5p inhibits the migration of metastatic melanoma cells at least in part by directly regulating the expression of IRS-2. The identity of other miR-7-5p target molecules that mediate its anti-migratory and anti-invasive effects on metastatic

melanoma cells, and studies to evaluate the anti-metastatic role of miR-7-5p in *in vivo* models of melanoma metastasis are the subject of further investigation.

These data support a role for miR-7-5p in the metastatic progression of melanoma. Downregulation of miR-7-5p in metastatic melanoma cells versus primary melanoma cells suggests that its loss may represent an early event in the metastatic process. miR-7-5p inhibits the metastatic processes of migration and invasion in multiple melanoma cell lines *in vitro*. Additionally, IRS-2, a known mediator of cell migration in other cancer systems, is a target for miR-7-5p in melanoma cell lines, where it may in part explain the anti-migratory effect of miR-7-5p. IRS-2 inhibition also reduces activity of Akt, a key oncogenic effector molecule in many cancers, including melanoma, suggesting that IRS-2 may represent a novel therapeutic target in some melanomas, with the potential to inhibit oncogenic PI3K/Akt signaling, cell migration and tumor metastasis. Our data provide further insight into the role of specific miRNAs in the progression of melanoma, and suggest that therapeutic upregulation of miR-7-5p might be of benefit in the treatment of this disease, possibly by delaying or preventing melanoma metastasis. Recent advances in miRNA delivery and targeting suggest that "miRNA replacement therapy" with miR-7-5p may be a feasible approach to cancer treatment [35].

Acknowledgments

The authors thank Dianne Beveridge for helpful comments, and acknowledge the funding support of the National Health and Medical Research Council, the Cancer Council of Western Australia, the Medical Research Commercialization Fund of Australia, and the Scott Kirkbride Melanoma Research Centre.

References

[1] K.T. Flaherty, Targeting metastatic melanoma, in: C.T. Caskey, C.P. Austin, J.A. Hoxie (Eds.), Annual Reviews, Palo Alto, U.S.A., Annu. Rev. Med. 63 (2012) 171-183.
[2] J.A. Sosman, K.B. Kim, L. Schuchter, R. Gonzalez, A.C. Pavlick, J.S. Weber, G.A. McArthur, T.E. Hutson, S.J. Moschos, K.T. Flaherty, P. Hersey, R. Kefford, D. Lawrence, I. Puzanov, K.D. Lewis, R.K. Amaravadi, B. Chmielowski, H.J. Lawrence, Y. Shyr, F. Ye, J. Li, K.B. Nolop, R.J. Lee, A.K. Joe, A. Ribas, Survival in BRAF V600–mutant advanced melanoma treated with vemurafenib, N. Engl. J. Med. 366 (2012) 707–714.
[3] N. Wagle, C. Emery, M.F. Berger, M.J. Davis, A. Sawyer, P. Pochanard, S.M. Kehoe, C.M. Johannessen, L.E. MacConaill, W.C. Hahn, M. Meyerson, L.A. Garraway, Dissecting therapeutic resistance to RAF inhibition in melanoma by tumor genomic profiling, J. Clin. Oncol. 29 (2011) 3085–3096.
[4] M.R. Fabian, N. Sonenberg, W. Filipowicz, Regulation of mRNA translation and stability by microRNAs, Annu. Rev. Biochem. 79 (2010) 351–379.
[5] V.F. Bonazzi, M.S. Stark, N.K. Hayward, MicroRNA regulation of melanoma progression, Melanoma Res. 22 (2012) 101–113.
[6] N.M.A. White, E. Fatoohi, M. Metias, K. Jung, C. Stephan, G.M. Yousef, Metastamirs: a stepping stone towards improved cancer management, Nat. Rev. Clin. Oncol. 8 (2011) 75–84.
[7] M.F. Segura, H.S. Greenwald, D. Hanniford, I. Osman, E. Hernando, MicroRNA and cutaneous melanoma: from discovery to prognosis and therapy, Carcinogenesis 33 (2012) 1823–1832.
[8] C. Migliore, A. Petrelli, E. Ghiso, S. Corso, L. Capparuccia, A. Eramo, P.M. Comoglio, S. Giordano, MicroRNAs impair MET-mediated invasive growth, Cancer Res. 68 (2008) 10128–10136.
[9] M. Garofalo, C.M. Croce, MicroRNAs: master regulators as potential therapeutics in cancer, in: A.K. Cho (Ed.), Annual Reviews, Palo Alto, Annu. Rev. Pharmacol. Toxicol. 51 (2011) 25-43.
[10] S.D.N. Reddy, K. Ohshiro, S.K. Rayala, R. Kumar, MicroRNA-7, a homeobox D10 target, inhibits p21-activated kinase 1 and regulates its functions, Cancer Res. 68 (2008) 8195–8200.
[11] B. Kefas, J. Godlewski, L. Comeau, Y. Li, R. Abounader, M. Hawkinson, J. Lee, H. Fine, E.A. Chiocca, S. Lawler, B. Purow, MicroRNA-7 inhibits the epidermal growth factor receptor and the Akt pathway and is down-regulated in glioblastoma, Cancer Res. 68 (2008) 3566–3572.
[12] L. Jiang, X. Liu, Z. Chen, Y. Jin, C.E. Heidbreder, A. Kolokythas, A. Wang, Y. Dai, X. Zhou, MicroRNA-7 targets IGF1R (insulin-like growth factor 1 receptor) in tongue squamous cell carcinoma cells, Biochem. J. 432 (2010) 199–205.
[13] D. Wu, Y. Wang, L. Fan, H. Luo, B. Han, L. Sun, X. Wang, J. Zhang, L. Cao, X. Wang, Y. You, N. Liu, MicroRNA-7 regulated glioblastoma cell invasion via targeting focal adhesion kinase expression, Chin. Med. J. 124 (2011) 2616–2621.
[14] I.P. Pogribny, J.N. Filkowski, V.P. Tryndyak, A. Golubov, S.I. Shpyleva, O. Kovalchuk, Alterations of microRNAs and their targets are associated with acquired resistance of MCF-7 breast cancer cells to cisplatin, Int. J. Cancer 127 (2010) 1785–1794.
[15] F.C. Kalinowski, K.M. Giles, P.A. Candy, A. Ali, C. Ganda, M.R. Epis, R.J. Webster, P.J. Leedman, Regulation of epidermal growth factor receptor signaling and erlotinib sensitivity in head and neck cancer cells by miR-7, PLoS ONE 7 (2012) e47067.
[16] D. Philippidou, M. Schmitt, D. Moser, C. Margue, P.V. Nazarov, A. Muller, L. Vallar, D. Nashan, I. Behrmann, S. Kreis, Signatures of microRNAs and selected microRNA target genes in human melanoma, Cancer Res. 70 (2010) 4163–4173.
[17] D.W. Mueller, A.K. Bosserhoff, Role of miRNAs in the progression of malignant melanoma, Br. J. Cancer 101 (2009) 551–556.
[18] D.J. Giard, S.A. Aaronson, G.J. Todaro, P. Arnstein, J.H. Kersey, H. Dosik, W.P. Parks, In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors, J. Natl. Cancer Inst. 51 (1973) 1417–1423.
[19] M. Herlyn, G. Balaban, J. Bennicelli, D. Guerry, R. Halaban, D. Herlyn, D.E. Elder, G.G. Maul, Z. Steplewski, P.C. Nowell, W.H. Clark, H. Koprowski, Primary melanoma cells of the vertical growth phase: similarities to metastatic cells, J. Natl. Cancer Inst. 74 (1985) 283–289.
[20] R.N. Fabricant, J.E. De Larco, G.J. Todaro, Nerve growth factor receptors on human melanoma cells in culture, Proc. Nat. Acad. Sci. U.S.A. 74 (1977) 565–569.
[21] X. Wang, B. Seed, A PCR primer bank for quantitative gene expression analysis, Nucleic Acids Res. 31 (2003) e154.
[22] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method, Methods 25 (2001) 402–408.
[23] K. Giles, A. Barker, P.M. Zhang, M.R. Epis, P.J. Leedman, MicroRNA regulation of growth factor receptor signaling in human cancer cells, Methods Mol. Biol. 676 (2011) 147–163.
[24] R. Limame, A. Wouters, B. Pauwels, E. Fransen, M. Peeters, F. Lardon, O. De Wever, P. Pauwels, Comparative analysis of dynamic cell viability, migration and invasion assessments by novel real-time technology and classic endpoint assays, PLoS ONE 7 (2012) e46536.
[25] X. Kong, G. Li, Y. Yuan, Y. He, X. Wu, W. Zhang, Z. Wu, T. Chen, W. Wu, P.E. Lobie, T. Zhu, MicroRNA-7 inhibits epithelial-to-mesenchymal transition and metastasis of breast cancer cells via targeting FAK expression, PLoS ONE 7 (2012) e41523.
[26] X. Zhao, W. Dou, L. He, S. Liang, J. Tie, C. Liu, T. Li, Y. Lu, P. Mo, Y. Shi, K. Wu, Y. Nie, D. Fan, MicroRNA-7 functions as an anti-metastatic microRNA in gastric cancer by targeting insulin-like growth factor-1 receptor, Oncogene (2012).
[27] Y.X. Fang, J.-L. Xue, Q. Shen, J. Chen, L. Tian, miR-7 inhibits tumor growth and metastasis by targeting the PI3K/AKT pathway in hepatocellular carcinoma, Hepatology (2012), http://dx.doi.org/10.1002/hep.25576 [Epub ahead of print].
[28] R.J. Webster, K.M. Giles, K.J. Price, P.M. Zhang, J.S. Mattick, P.J. Leedman, Regulation of epidermal growth factor receptor signaling in human cancer cells by microRNA-7, J. Biol. Chem. 284 (2009) 5731–5741.
[29] O. Saydam, O. Senol, T. Würdinger, A. Mizrak, G.B. Ozdener, A.O. Stemmer-Rachamimov, M. Yi, R.M. Stephens, A.M. Krichevsky, N. Saydam, G.J. Brenner, X.O. Breakefield, MiRNA-7 attenuation in schwannoma tumors stimulates growth by upregulating three oncogenic signaling pathways, Cancer Res. 71 (2011) 852–861.
[30] J.-C. Marshall, A. Nantel, P. Blanco, J. Ash, S. Cruess, M. Burnier, Transcriptional profiling of human uveal melanoma from cell lines to intraocular tumors to metastasis, Clin. Exp. Metastasis 24 (2007) 353–362.
[31] K. Mardilovich, S. Pankratz, L. Shaw, Expression and function of the insulin receptor substrate proteins in cancer, Cell. Signal. 7 (2009) 14.
[32] L.M. Shaw, Identification of insulin receptor substrate 1 (IRS-1) and IRS-2 as signaling intermediates in the $\alpha 6\beta 4$ integrin-dependent activation of phosphoinositide 3-OH kinase and promotion of invasion, Mol. Cell. Biol. 21 (2001) 5082–5093.
[33] R.A. Shivdasani, MicroRNAs: regulators of gene expression and cell differentiation, Blood 108 (2006) 3646–3653.
[34] S.V. Madhunapantula, G.P. Robertson, Therapeutic implications of targeting Akt signaling in melanoma, Enzyme Res. 2011 (2011) 1–20.
[35] A.G. Bader, D. Brown, M. Winkler, The promise of microRNA replacement therapy, Cancer Res. 70 (2010) 7027–7030.