

Searching for the ‘melano-miRs’: miR-214 drives melanoma metastasis

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Since the beginning of micro-RNA (miR) research, several attempts have been made to identify the ‘melano-miRs’, which are involved in melanoma progression. Indeed, a small number of miRs have been identified to regulate some genes involved in melanogenesis. However, the miRs that control the pathway to the malignant phenotype are yet undescribed. In this issue of the *EMBO Journal*, Penna *et al* (2011) demonstrate that miR-214 is overexpressed in metastatic melanoma cell lines and tumour specimens. miR-214 regulates the expression of two transcription factors AP-2 γ (directly) and AP-2 α (indirectly). These transcription factors, particularly AP-2 α , have been previously shown to play major roles in melanoma metastasis via regulation of genes involved in extravasation, invasion and angiogenesis. As such, this study has identified miR-214 as a driver of melanoma metastasis.

Limited data are available about the changes in miRNA expression levels in malignant melanoma. This investigative effort began in 2008 with the first report by Bemis *et al* (2008), linking the expression of a single miR, miR-137, with the expression of MITF, a ‘master regulator’ of melanogenesis. MITF, however, has since been shown to be also regulated by miR-182 (Segura *et al*, 2009). Overexpression of miR-182 was associated with increased survival and invasive properties of melanoma cells via regulation of both MITF and FOXO3. miR-182 was found to enhance the invasive capability

of melanoma cells *in vitro* and increase their metastatic potential *in vivo* in an experimental lung metastasis model. However, these studies never identified the ‘metastatic’ target genes involved in this process. Other miRNAs with known target genes in melanoma are miR-221 and miR-222, which regulate the expression of both p27 and in particular c-KIT, which is downregulated in metastatic melanoma cells. The *c-KIT* gene is regulated mainly by the AP-2 α transcription factor (*vide infra*). Moreover, treatment with antagomiRs against miR-221/222 inhibited melanoma proliferation and invasion *in vitro* and melanoma growth *in vivo* (Felicetti *et al*, 2008). The first discovered miR to be involved in cancer formation was miRNA let-7. The family of miRs let 7a and let 7b were later reported to play a role in melanoma tumourigenesis. For example, let 7b acts as a negative regulator of melanoma cell proliferation via regulation of cyclin D1, whereas let 7a was demonstrated to regulate the expression of integrin- β_3 and the Ras Oncogene (reviewed in Mueller and Bosserhoff, 2009).

All of the above studies focused on single miRs and their individual roles in melanoma progression. Other attempts have been made to identify a cluster of miRs that are either involved in melanogenesis or predictors of survival. For example, Ozsolak *et al* (2008) searched for miRs that are specific to melanoma progression. To that end, they screened for miRs that are specifically regulated by MITF and identified

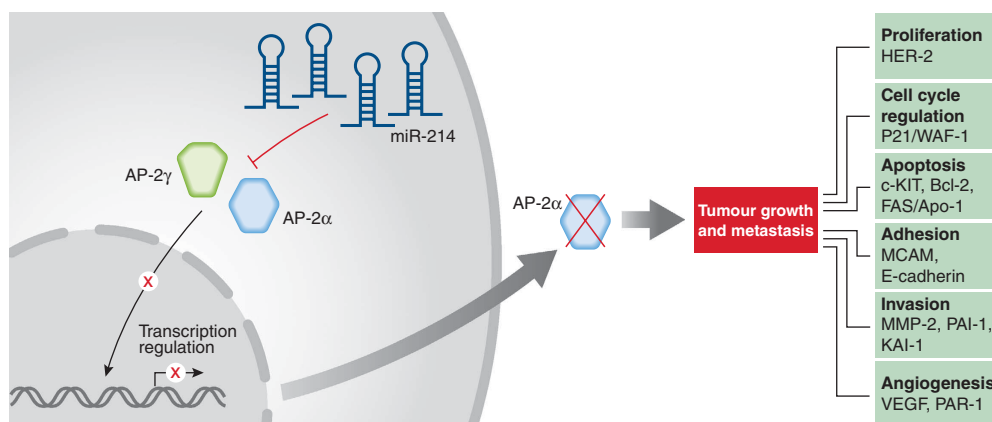


Figure 1 Proposed model for how miR-214 drives melanoma metastasis. miR-214 post-transcriptionally downregulates the expression of AP-2 γ and AP-2 α . In turn, AP-2 α functions as a master switch regulating a cascade of genes involved in melanoma metastasis, including genes regulating proliferation (HER-2), cell cycle (p21), apoptosis (c-KIT, Bcl-2, Fas/APO-1), adhesion (MCAM/MUC18, E-cadherin), invasion (MMP-2, PAI-1, KAI-1) and angiogenesis (VEGF, PAR-1).

miR-146a, miR-221/222 and miR-363. Another approach pursued miRNAs silenced by CpG methylation, revealing miR34a to be highly methylated in melanoma cell lines and primary tumours. Although the role of miR-34a in melanoma progression has yet to be elucidated, it was elsewhere reported that miR-34a regulates the expression of MET. While these studies concentrated on the biological functions of these miRNAs and their contribution to melanogenesis, other studies have attempted to identify miRNA signatures for diagnostic and prognostic purposes. In one such study, Segura *et al* (2010) analysed the expression of 611 miRNAs in 59 metastatic specimens with detailed clinical follow-up. They were able to identify a miRNA classifier consisting of six miRNAs (miR-150, miR-342-3p, miR-455-3p, miR-145, miR-155 and miR-497) that are predictors of post-recurrence survival, with an accuracy of about 80%. A slightly different approach has been taken by Caramuta *et al* (2010). This group analysed miR expression profiling in lymph node metastases and reported a unique signature consisting of downregulation of miR-191, combined with upregulation of miR-193a, miR-193b, miR-365, miR-338 and let-7. Together, this cluster serves as a predictor of short-term survival of melanoma patients. A broader approach has been more recently taken to characterize the melanoma miRNAome by deep sequencing. However, the identification of an exact, single miR or miRNAs signature that best predicts metastatic development has yet to be reported or validated. One such miR is described in this issue of the *EMBO Journal* by Penna *et al* (2011).

To identify miRNAs that are specifically affecting melanoma metastasis, Penna *et al* (2011) have utilized the parental A375P cell line and its metastatic variants that were selected via cycling in nude mice. Among the miRNAs that were differentially expressed in this model system, miR-214 was found to be overexpressed in the metastatic variants. Overexpression of miR-214 was further validated in metastatic lesions utilizing melanoma tissue array, thus, confirming its upregulation in 'real' life. The authors then re-expressed miR-214 in the low-metastatic variants (miR-214 negative) or silenced its expression via antagomiR in the highly metastatic cells, revealing its involvement in migration, invasion and extravasation *in vitro*. *In vivo*, miR-214 did not affect the growth of the primary tumours, but did enhance the metastatic potential of cells in an experimental

lung metastasis assay, thus, indicating the involvement of miR-214 in the transition point from RGP to VGP of melanoma progression. The novelty of this study, however, is emphasized when the authors identified the downstream target genes for miR-214. These include the transcription factor AP-2 γ and the integrin- α_3 (ITGA3). Importantly, another family member, the AP-2 α transcription factor, was also affected by miR-214. It is not clear, however, at this step whether the downregulation of AP-2 α in the metastatic melanoma cells resulted from a direct effect of miR-214 overexpression or by its regulation by AP-2 γ . The authors, in this case, analysed the expression of AP-2 α after miR-214 overexpression in metastatic melanoma cells endogenously harbouring low levels of AP-2 α .

The expression of the AP-2 α transcription factor has been shown to be downregulated in metastatic melanoma cell lines and tumour specimens (reviewed in Bar-Eli, 1999). Loss of AP-2 α drives the melanoma metastatic phenotype by regulating a cascade of genes, such as c-KIT, MCAM/MUC18 (one of the target genes identified by Penna *et al* (2011)), HER-2, VEGF, MMP-2 and the thrombin receptor PAR-1, all of which were shown to play a role in melanoma metastasis (Figure 1). PAR-1 activation, in turn, results in the expression and secretion of proangiogenic factors, such as IL-8, PDGF and VEGF. PAR-1 has also recently been shown to regulate the expression of the tumour suppressor gene, Maspin, in melanoma (Villares *et al*, 2011). As such, AP-2 α plays a pivotal role in melanoma metastasis. However, up until this point, the mechanism(s) for its loss of expression in metastatic melanoma cells have not been known. The study by Penna *et al* (2011) provides the missing piece of the puzzle: the identification of miR-214 as a post-transcriptional regulator of AP-2 α , to drive melanoma metastasis. By affecting the expression of AP-2 α and its downstream target genes, miR-214 is largely involved in modulating the melanoma tumour microenvironment to enhance invasion and metastasis. It will be interesting now to determine whether overexpression of miR-214 could be used for prognostic purposes and more importantly, whether its targeting could provide a new treatment modality for melanoma.

Conflict of interest

The author declares that he has no conflict of interest.

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