Breast Cancer Online, 2008, **11**(1), Page 1 of 3, ***e2***
doi:10.1017/S1470903108006688

ISSN 1470-9031

Breast Cancer Online
www.bco.org

Journals Club

Review of: Tumour invasion and metastasis initiated by microRNA-10b in breast cancer

Alex Swarbrick

Cancer Research Program, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia.

Citation of the original article:
L. Ma, J. Teruya-Feldstein, R. A. Weinberg. ***Nature*** 2007; **449**(7163): 682–688; Epub 26 September 2007.

Abstract of the original article:
MicroRNAs have been implicated in regulating diverse cellular pathways. Although there is emerging evidence that some microRNAs can function as oncogenes or tumour suppressors, the role of microRNAs in mediating cancer metastasis remains unexplored. Here we show, using a combination of mouse and human cells, that microRNA-10b (miR-10b) is highly expressed in metastatic breast cancer cells and positively regulates cell migration and invasion. Overexpression of miR-10b in otherwise non-metastatic breast tumours initiates robust invasion and metastasis. Expression of miR-10b is induced by the transcription factor Twist, which binds directly to the putative promoter of miR-10b (MIRN10B). The miR-10b induced by Twist proceeds to inhibit translation of the messenger RNA encoding homeobox D10, resulting in increased expression of a well-characterized pro-metastatic gene, RhoC. Significantly, the level of miR-10b expression in primary breast carcinomas correlates with clinical progression. These findings suggest the workings of an undescribed regulatory pathway, in which a pleiotropic transcription factor induces expression of a specific microRNA, which suppresses its direct target and in turn activates another pro-metastatic gene, leading to tumour cell invasion and metastasis.

Review

Despite the importance of metastasis to breast cancer survival and morbidity, our understanding of the cellular and molecular mechanisms underpinning breast cancer metastasis is formative. While it is still debated whether the metastatic spread of a tumour is controlled by 'metastasis genes' distinct from those driving primary tumourigenesis, recent studies have identified genes that regulate organ-specific metastasis of experimental breast cancer models (see [1–3] as examples). In their recent paper in Nature, Ma *et al.* [4] add a twist to this list of metastatic regulators by describing a role for the miR-10b microRNA in the control of breast cancer invasion and metastasis.

MicroRNAs are small regulatory RNAs that control gene expression by repressing the translation and/or enhancing the degradation of target mRNAs through a process known as RNA interference (RNAi) [5]. Discovered in 1993 in nematodes, microRNAs have revolutionised our view of cell and molecular biology as they control numerous processes including many aspects of embryonic development and disease states including cancer [6]. Expressed as precursor

Correspondence to: Alex Swarbrick, Cancer Research Program, Garvan Institute of Medical Research, Darlinghurst, NSW 2010, Australia. E-mail: a.swarbrick@garvan.org.au; Tel: +61 2 9295 8378; Fax: +61 2 9295 8321

Received: 04/01/08
Accepted: 07/01/08
BCO/668/2007/JC

RNAs either from their own promoters or within introns of other genes, they are enzymatically processed to the mature (~20–30 nucleotide) active microRNA.

Due to the promiscuous nature of their binding to target mRNAs, microRNAs are thought to control the expression of many target genes, thus acting to integrate large-scale gene expression programs. The practical implication of this promiscuity is that identifying the key targets of microRNAs in a given process is far from facile. In this paper, Ma *et al.* make the extraordinary achievement of identifying not only a new role for microRNAs in metastasis but also the downstream targets of this microRNA and the upstream transcription factor that directs the expression of this microRNA.

The story begins with Ma *et al.* investigating a panel of candidate microRNAs previously identified as altered between normal breast tissue and breast cancer samples [7]. After examining the expression of eight microRNAs across a panel of immortalised or transformed breast epithelial cell lines, they found that three (miR-155, miR-9 and miR-10b) were upregulated in transformed cells. Interestingly, miR-10b was highly expressed only in metastatic cancer lines.

To investigate further, Ma *et al.* then used in vitro assays with cell lines in which they manipulated the expression of miR-10b. They showed that while miR-10b had no impact on cellular proliferation or death, it was both sufficient and necessary for the invasive behaviour of breast cancer cell lines in vitro. They then tested this result in vivo by transplanting poorly invasive breast cancer cell lines into the cleared mammary fat pad of immune-deficient mice. While control cells formed well-encapsulated tumours, cells overexpressing miR-10b invaded the fat pad, entered blood vessels and within 6 weeks formed distant metastases in the lung and peritoneum of their hosts. Interestingly, both groups of tumours grew at approximately the same rate. Thus, in vivo, as in vitro, miR-10b plays a role in metastatic progression but not primary tumour growth and may indeed be a 'metastasis gene'.

To understand how miR-10b might be regulated during tumour progression, Ma *et al.* turned to a transcription factor previously identified as a gene driving epithelial to mesenchymal transdifferentiation (EMT) and metastasis: Twist [3]. In a series of elegant experiments in vitro, they showed that when overexpressed, Twist directly binds upstream of the MIRN10B gene and promotes its expression. Intriguingly, blocking this increase in miR-10b impinged only upon the ability of Twist to drive invasion, whereas cells still underwent EMT. Thus miR-10b is induced by Twist and mediates its effects on invasion, not EMT.

Ma *et al.* then turned their attention to identifying the mRNAs regulated by miR-10b. From the ~100 mRNAs computationally predicted to be targets of miR-10b, they focussed their attention to the homeobox D10 gene (*HOXD10*), which has been implicated previously in the suppression of invasion and migration. They showed that overexpression of miR-10b repressed the translation of *HOXD10* mRNA. Not happy to rest at this result, the authors set out to identify the effector/s downstream of Hoxd10, since others have previously shown the regulation by Hoxd10 of several factors controlling cellular migration. They observed that in cells overexpressing miR-10b, Hoxd10 levels decline and the expression of one of these factors, RhoC, increases. Further, overexpression of Hoxd10 or depletion of RhoC is sufficient to block the pro-invasive effects of miR-10b overexpression.

Taken together, these studies define a pathway from elevated levels of the transcription factor Twist, which upregulates the microRNA miR-10b. miR-10b then binds to the *HOXD10* mRNA, repressing its translation. Finally, reduced Hoxd10 protein leads to increased RhoC and cellular migration/invasion.

Fascinatingly, the MIRN10B gene is located within a cluster of *HOX* genes, including *HOXD10*, suggesting that this pathway may have evolved to function in a coordinated manner. However, MIRN10B is in a different location within the mouse and human *HOX* clusters. It is not clear whether Twist binds to the same regulatory elements upstream of human and murine MIRN10B, even though mouse and human cells are used interchangeably for some of the cell biology and gene expression analyses reported.

Finally, the authors test the relevance of these findings to human disease. Across a panel of breast cancers, 5/5 women with non-metastatic disease had lower miR-10b expression in their tumours than matched normal controls, while ~50% of women with metastatic disease had elevated levels of miR-10b in their primary tumour. As with this group's previous study of Twist [3], perhaps the weakest aspect of this report is its clinical data. The current study evaluates miR-10b expression in only 23 human breast cancers of unspecified histological subtype and gene expression category. It is not reported whether any correlations between Twist, miR-10b, HoxD10 and RhoC expression are found. Furthermore, the expression of miR-10b between the non-metastatic and highly metastatic patients barely reaches statistical significance, presumably due to the small sample sizes and/or technical issues. Given that a previous study conflictingly identified miR-10b as downregulated in breast cancer compared to normal [7], further work is required to test the validity of these findings in clinical specimens.

https://doi.org/10.1017/S1470903108006688 Published online by Cambridge University Press

The primary importance of this study is that it is the first demonstration of a role for microRNAs in metastatic progression and also provides further evidence for the existence of genes whose role in cancer is confined to regulation of metastatic success.

References

1. Minn AJ, Teruya-Feldstein J, Weinberg RA. Genes that mediate breast cancer metastasis to lung. *Nature* 2005; **436**: 518–524.
2. Kang Y, Siegel PM, Shu W, *et al*. A multigenic program mediating breast cancer metastasis to bone. *Cancer Cell* 2003; **3**: 537–549.
3. Yang J, Mani SA, Donaher JL, *et al*. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 2004; **117**: 927–939.
4. Ma L, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature* 2007; **449**: 682–688.
5. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281–297.
6. Kent OA, Mendell JT. A small piece in the cancer puzzle: microRNAs as tumor suppressors and oncogenes. *Oncogene* 2006; **25**: 6188–6196.
7. Iorio MV, Ferracin M, Liu CG, *et al*. MicroRNA gene expression deregulation in human breast cancer. *Cancer Res* 2005; **65**: 7065–7070.

https://doi.org/10.1017/S1470903108006688 Published online by Cambridge University Press