MicroRNA-10b and breast cancer metastasis

Arising from: L. Ma, J. Teruya-Feldstein & R. A. Weinberg *Nature* **449**, 682–688 (2007)

MicroRNAs regulate messenger RNA expression but are frequently dysregulated in tumours. Ma *et al.*[1] report that overexpression of microRNA-10b (miR-10b) initiates invasion and metastasis in models of breast cancer and that its expression in primary breast carcinomas correlates with clinical progression. We tested this in patients with primary breast cancer, of whom 92 had nodal metastases at diagnosis and 127 were node-negative. We found no significant association between miR-10b levels and metastasis or prognosis. Although we concede that miR-10b may have a biological effect in a few cells at the growing edge of a tumour, we believe that it is unlikely to correlate in whole tumour samples with clinical progression.

Ma *et al.*[1] found that miR-10b was upregulated in metastatic breast cancer cell lines compared with primary human mammary epithelial cells or spontaneously immortalized MCF-10A cells, and that its overexpression initiated invasion and metastasis in xenograft models of breast cancer. Their Fig. 6 (ref. 1) shows increased miR-10b in tumours of 9 out of 18 metastasis-positive patients, compared with none of 5 patients without metastasis—hence their conclusion that miR-10b is associated with metastasis outcome. However, their patient group was small, the type of metastasis not defined, and data on clinical variables, age and follow-up limited.

Ma *et al.*[1] converted the relative amount of miR-10b in tumour samples, a continuous variable, into a series of discrete variables and then applied a chi-squared test, applicable to categorical variables, instead of a non-parametric Mann–Whitney test.

We studied 219 consecutive patients with early breast cancer, for whom fresh frozen samples and long-term follow-up were available. We examined the population-based outcome, following the REporting recommendations for tumour MARKer prognostic studies (REMARK)[2], measuring expression of miR-10b by real-time PCR and normalizing our values to three controls—the small nucleolar RNAs RNU 43, RNU 44 and RNU 48—and to normal tissue.

We also found less miR-10b expression in patients without metastasis ($n = 114$) than in normal breast tissue ($n = 10$). However, unlike Ma *et al.*[1], we found lower miR-10b expression in patients with distant relapse ($n = 61$), regional relapse ($n = 11$) and local recurrence ($n = 33$). If miR-10b has a role in metastasis, we would expect a positive correlation with factors such as the presence or number of tumour-involved lymph nodes at the time of diagnosis; however, no significant correlation was found (Table 1). MiR-10b expression did not correlate with development of distant metastases, recurrence-free survival or distant-relapse-free survival[3], or with recurrence-free survival and breast-cancer-specific survival[4] (Fig. 1). Whereas Ma *et al.*[1] found that miR-10b overexpression increases tumour size and invasiveness, we find that its expression correlates inversely and significantly with tumour size and grade.

Table 1 | Relationship between miR-10b, miR-210 and clinico-pathological variables

	Correlation with clinico-pathological variables	
	miR-210	miR-10b
Categorical variable		
Nodal status* (node-positive = 92; node-negative = 127)	$Z = -1.13$, $P = 0.26$	$Z = -0.52$, $P = 0.61$
Grade†	$\chi^2 = 6.66$, $P = 0.04$	$\chi^2 = 9.40$, $P = 0.01$
Continuous variable‡		
ER ELISA	$\rho = -0.11$, $P = 0.11$	$\rho = 0.09$, $P = 0.19$
Tumour size (cm)	$\rho = 0.08$, $P = 0.22$	$\rho = -0.20$, $P = 0.003$

ER, oestrogen receptor.
* Mann–Whitney test.
† Chi-squared test.
‡ Spearman rank test.

In the same patient cohort, increased expression of hypoxia-induced microRNA miR-210 correlated with poor prognosis[5]. Our negative result for miR-10b is therefore unlikely to be due to problems of extraction, stability or analysis. Although the RNU6B control used by Ma *et al.*[1] is reported to vary more between tissues than RNU 44 or RNU 48 (ref. 6), we found that the four control genes had comparable stability in a subset of 48 samples and 5 pooled controls.

Our results are supported by microRNA array data. Downregulated miR-10b expression in breast carcinoma[7] was found to be associated with vascular invasion but not with oestrogen-receptor status or nodal involvement. Expression profile comparison of microRNAs and mRNAs in primary breast cancers indicated that miR-10b is associated with the prognostically favourable 'luminal A' subtype[8].

We conclude that miR-10b overexpression does not correlate with distant metastases or poor prognosis in breast cancer. Studies using many more patients with full clinicopathological information are needed before conclusions can be drawn about the function of miR-10b.

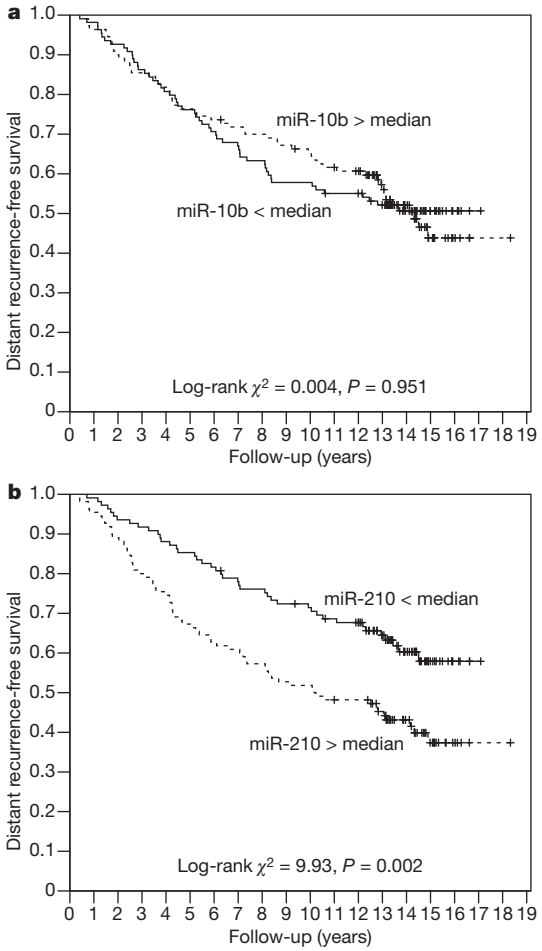

Figure 1 | miR-10b is not significantly associated with metastasis. Kaplan–Meier curves showing the relationship of miR-10b (**a**) and miR-210 (**b**) with distant recurrence-free survival (as defined by STEEP criteria[3]) of 219 patients with breast cancer. The results of a log-rank test are shown; miR-210 and miR-10b expression levels were stratified by median values. *P* values are computed by the use of log-rank test to miR-210 and miR-10b expression levels stratified by median values.

METHODS

A group of 219 patients with early first primary breast cancer, treated in Oxford from 1989 to 1992, were studied (ethical approval from the local Research Ethics Committee). RNA was extracted from liquid-nitrogen-frozen breast tumour samples or normal breast tissue using Tri-reagent (Sigma-Aldrich). MicroRNA expression was assessed by real-time PCR with TaqMan MicroRNA assay protocol (Applied Biosystems) using 5 ng total RNA per gene (for further details and methods, see ref. 5). Fold changes in microRNA expression were determined by the $2^{-\Delta\Delta Ct}$ method, normalizing the results to normal breast tissue and expression of RNU 43, RNU 44 and RNU 48.

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Ma et al. *reply*

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Gee *et al.* contest that microRNA-10b is not a prognostic marker for metastasis risk in breast cancer[1]. However, their observations do not bear on the pro-metastatic roles of microRNA-10b (miR-10b) as we described them[2], nor do they undermine our conclusions[1].

We used a variety of human metastatic cancer lines and tumours to discover which microRNA, if any, was correlated with metastatic behaviour, rather than to find a prognostic clinical marker. Our functional analysis of miR-10b suggested that it is mechanistically important[2], but we have no idea whether its expression in early-stage whole-tumour specimens can predict clinical progression. The discovery by Gee *et al.*[1] that miR-10b is not a useful prognostic marker is therefore not surprising.

Whereas Gee *et al.*[1] studied early-stage cancers, we analysed 23 primary tumour samples obtained at the time of mastectomy, when both lymph node and distant metastases had already developed. On the basis of these findings[2], we concluded that miR-10b expression increased in metastatic breast tumours—not that its expression in unfractionated bulk populations of tumours could predict metastatic relapse[2]. MiR-10b is also significantly upregulated in human glioblastoma multiforme[3] and in pancreatic adenocarcinomas[4], two types of highly invasive and/or metastatic cancers.

Our results indicate that induction of miR-10b expression is probably not an early event during tumour progression, but occurs after activation of the Twist transcription factor at a later stage[2]. We propose that expression of Twist and miR-10b in many primary tumours is likely to be only local and transient, occurring in a minority of cells such as those in the invasive front of a breast tumour that are responding to contextual signals from nearby stromal cells to undergo an epithelial–mesenchymal transition. However, it is technically difficult to capture this minority population of cancer cells. Almost all microRNA expression analyses performed so far have been on bulk individual tumours consisting of neoplastic cells and recruited non-neoplastic stromal cells. These are therefore liable to underestimate or even misinterpret the range of messenger RNAs and microRNAs expressed by a small minority of the cells within such tumours.

Meaningful clinical data may eventually come from determination of which types of primary tumour cell do and do not express Twist and miR-10b when exposed to certain heterotypic contextual signals of stromal origin. Tumours that behave badly clinically might be those that upregulate Twist and miR-10b in response to such signals, but this is still years away from miR-10b as a prognostic marker. We agree with Gee *et al.*[1] that sufficient numbers of patients with long-term follow-up are needed for clinical correlative studies of molecular markers in breast cancer and other diseases.

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