MicroRNAs: critical regulators of epithelial to mesenchymal (EMT) and mesenchymal to epithelial transition (MET) in cancer progression

Marc D. Bullock1, Abdulkadir E. Sayan, Graham K. Packham and Alex H. Mirnezami

Cancer Research UK Centre, Somers Cancer Research Building, University of Southampton Cancer Sciences Division, Southampton University Hospital NHS Trust, Tremona road, Southampton, SO16 6YD, United Kingdom

MicroRNAs (miRNAs) are a class of small highly conserved RNAs that provide widespread expressional control through the translational repression of mRNA. MiRNAs have fundamental roles in the regulation of intracellular processes, and their importance during malignant transformation and metastasis is becoming increasingly well recognized. An important event in the metastatic cascade is epithelial to mesenchymal transition (EMT), a reversible phenotypic switch over, which endows malignant epithelial cells with the capacity to break free from one another and invade the surrounding stroma. Our understanding of EMT has been significantly improved by the characterization of miRNAs that influence the signalling pathways and downstream events that define EMT on a molecular level.

Here, we detail the role of miRNAs in EMT, and in doing so demonstrate their importance in the early stages of the metastatic cascade; we discuss a significant body of data that suggest new opportunities for drug development, and we highlight critical knowledge gaps that remain to be addressed.

Introduction: metastasis and EMT
Metastasis is a complex, multi-step and dynamic biological event. To succeed in this process, cancer cells need to acquire the capacity to break free from their sister cells, invade the extracellular matrix (ECM) and basement membrane (BM), intravasate into the circulation, evade immune detection and eventually extravasate and propagate at distant sites, establishing viable secondary tumour deposits in remote organ systems (Coghlin and Murray, 2011). This elaborate process is responsible for the vast burden of cancer-associated morbidity and mortality. Recognition of this has provoked an increasing amount of research activity into the pathogenesis and treatment of metastasis, and brought into sharp focus the molecular mechanisms that underlie cancer progression.

One mission-critical step in the metastatic cascade is the process of epithelial to mesenchymal transition (EMT). EMT is an evolutionarily conserved program of gene expression during which epithelial cells adopt characteristics of mesenchymal cells. While the significance of EMT in gastrulation and neural crest cell migration during embryogenesis has long been established, in more recent times EMT has also provided important insights into how during the course of malignant transformation, polarized epithelial cells, bound firmly to the BM and to one another, acquire mesenchymal attributes such as enhanced motility, resistance to apoptosis and the capacity to invade the surrounding stroma (Thiery, 2003). EMT is regulated by a variety of signalling pathways originating from the stroma surrounding cancer cells,
including Transforming Growth Factor-beta (TGFβ), Hepatocyte Growth Factor (HGF), Platelet Derived Growth Factor (PDGF), Epidermal Growth Factor (EGF) and Integrin engagement, all of which converge at the level of key transcription factors such as ZEB, SNAIL and TWIST (Kalluri and Weinberg, 2009). Activation of these transcription factors subsequently brings about repression of epithelium-specific gene expression including cytoskeletal and cell surface proteins (Batlle et al., 2000; Comijn et al., 2001; Yang et al., 2004; Imamichi et al., 2007; Kokudo et al., 2008; Medici et al., 2008; Thiery et al., 2009). Epithelial cells are typically characterized by expression of E-cadherin and cytokeratins, and loss of expression of these markers is a key hallmark of EMT. E-cadherin and cytokeratin are downregulated in mesenchymal cells, and replaced during EMT by the mesenchymal specific markers vimentin and fibronectin (Kalluri and Weinberg, 2009; Iwatsuki et al., 2010).

In more recent years, the mechanisms behind EMT have been increasingly laid bare, with the pathways involved in downregulation of epithelial markers and corresponding upregulation of mesenchymal markers progressively better characterized. One area of significant progress has been the identification of the critical role played by microRNAs (miRNAs) in these processes. In the present review, we detail the recent advances in this field and explore well established but evolving concepts in EMT. We discuss a significant body of new data not previously featured in review format, and highlight critical knowledge gaps that remain to be addressed to enable improved understanding of the molecular mechanisms behind EMT and metastasis.

MiRNAs
MiRNAs are a class of small, highly conserved non-coding RNAs that provide widespread control of gene expression through translational repression of mRNA. MiRNAs have fundamental roles in the regulation of cellular processes and are increasingly recognized to play a critical role in malignant transformation, with a growing number of onco-genes and tumour suppressor genes found to be under miRNA control (Iorio and Croce, 2009). MiRNA genes account for 2–5% of the human genome, and are commonly clustered within the introns of protein coding genes (Rodriguez et al., 2004). Primary miRNA transcripts (pri-miRNAs) are generated by RNA polymerase II and processed into 70-nucleotide stem-loop structures called pre-miRNAs by the ribonuclease Drosha. Pre-miRNAs undergo further processing by the cytoplasmic endonuclease enzyme Dicer to form mature, 18–25 nucleotide miRNAs. One strand of the mature miRNA becomes incorporated into the RNA-induced silencing complex (RISC), and through partial sequence complementarity interacts with the 3’ untranslated regions (UTR) of target mRNAs to bring about translational repression or mRNA degradation, in a manner comparable to exogenous short interfering RNAs (siRNAs) (Bartel, 2009).

Over 1400 miRNAs have now been identified in humans, and de-regulation of miRNAs has profound consequences, as each individual miRNA promiscuously targets multiple genes and is capable of inducing broad downstream and feedback effects simultaneously. The notion that miRNAs are also powerful regulators of EMT has transformed the conventional narrative of carcinoma progression, and several miRNAs have now been described as crucial regulators in EMT. These miRNAs dynamically influence the balance between EMT and the reverse process termed mesenchymal to epithelial transition (MET) (Gibbons et al., 2009), and will form the basis of the review presented here. In addition, miRNAs link EMT with other important strands of research, including chemotherapy resistance and the role of cancer stem cells, providing new insights into the nature of malignant cells capable of completing the metastatic journey and uncovering fertile ground for future drug development.

TGFβ induced miRNA expression during EMT
EMT is induced in malignant epithelial cells by extracellular signals originating from tumour-associated stroma. TGFβ appears to play a dominant role, directly activating transcription factors ZEB, SNAIL and TWIST; the master regulators of EMT (Kalluri and Weinberg, 2009).

TGFβ is a facilitator of metastasis, however the role it plays in carcinogenesis overall is complex. TGFβ inhibits cellular proliferation and maintains epithelial differentiation in the early stages of
malignant transformation but in more advanced disease tumour suppressive responses are lost, with pro-invasive, pro-metastatic consequences (Massague, 2008).

TGF\(\beta\) binds the TGF\(\beta\) II receptor (T\(\beta\)RII), a serine/threonine receptor kinase, leading to recruitment of TGF\(\beta\) I receptor (T\(\beta\)RI) into a TGF\(\beta\)/T\(\beta\)R complex. Activated T\(\beta\)RI phosphorylates receptor-specific SMAD 2 and 3 elements that associate subsequently, with SMAD 4 to form a heteromeric complex capable of translocation into the nucleus and exerting transcriptional control over numerous genes including several involved in EMT activation (Zavadil and Bottinger, 2005).

A number of miRNA expression profiling studies have been conducted to identify candidate miRNAs with possible roles in TGF\(\beta\)-induced EMT (Figure 1) (Kong et al., 2008; Korpal et al., 2008; Gebeshuber et al., 2009; Cottonham et al., 2010; Eades et al., 2011).

MiR-21 and miR-31 are the most strongly induced miRNAs (between 2- and 3-fold) in response to simultaneous treatment of LIM1863 organoids with TGF\(\beta\) and TNF\(\alpha\). The LIM1863 cell culture system represents a model of colon cancer in three dimensions; Differentiated goblet and columnar cells, organized around a central lumen and grown in suspension can be made to undergo EMT, characterized by adoption of a monolayer morphology and adherence to the tissue culture plate, in response to TGF\(\beta\), a transition that can be accelerated by the addition of TNF\(\alpha\). Both miR-21 and miR-31 are capable of enhancing the invasive and migratory phenotype of LIM1863 cells and both negatively regulate the expression of TIAM1 (T-lymphoma invasion and metastasis 1) (Cottonham et al., 2010). TIAM1, a guanine nucleotide exchange factor (GEF) for the Rac GTPase has been implicated in regulation of cancer cell invasion in numerous studies, and miR-21/miR-31 over-expression is clinically a feature of late-stage colon cancer metastasis (Slaby et al., 2007). Transfecting LIM1863 cells with either miR-21 or miR-31 significantly reduces the abundance of TIAM1 protein, as demonstrated by western blotting and yet mRNA expression is unaffected. Together, this evidence suggests that in the context of colorectal cancer progression, miR-21 and miR-31 facilitate TGF\(\beta\)-induced EMT by targeting TIAM1, repressing its translation rather than inducing mRNA degradation (Cottonham et al., 2010).

Using the murine mammary EpH4/EpRas/RasXT cell culture system in which parent cells transit from an epithelial phenotype to a metastatic mesenchymal phenotype in response to TGF\(\beta\), Gebeshuber and colleagues identified that the most highly upregulated miRNA during EMT was miR-29a (4.7-fold). EpRas cells have an epithelial phenotype and are derived from non-tumourogenic EpH4 cells. They become tumourigenic because of constitutive over-expression of the oncogene H-Ras-V12. EpRas cells can undergo EMT and metastasise in response to TGF\(\beta\) forming RasXT cells that stably maintain a mesenchymal phenotype.

MiR-29a was subsequently shown to target the expression of the regulatory protein tristetraproline (TTP) and downregulate its expression by approximately 60%, leading to disruption of epithelial polarity in vitro and enhanced metastasis formation in mouse models. In vivo, high miR-29a expression and low TTP expression is a consistent feature of invasive ductal carcinoma, but in individual samples, their correlation was only slightly negative, emphasizing that miRNA expression control is only one part of a broader regulatory apparatus (Gebeshuber et al., 2009).

MiR-155 is another miRNA over-expressed in several malignancies (Eis et al., 2005; Iorio et al., 2005), and has been shown to be a direct transcriptional target of the TGF\(\beta\)/SMAD4 signalling cascade. Kong and co-workers demonstrated that miR-155 was significantly over-expressed in normal murine
mammary epithelial cells (NMuNG) treated with TGFβ compared with SMAD4-knockdown NMuNG cells subjected to the same treatment. MiR-155 promotes EMT by targeting RhoA GTPase, an important regulator of cellular polarity and tight junction formation and stability. MiR-155 knockdown suppresses TGFβ-induced EMT and although ectopic expression alone is insufficient to induce EMT, miR-155 over-expression does promote mesenchymal characteristics such as disruption of cellular adhesion and enhanced cellular motility and invasion. Importantly, the restoration of RhoA using an expression vector cloned without its 3’ UTR eliminates these changes even in the presence of ectopic miR-155 (Kong et al., 2008).

In further studies using NMuNG murine mammary epithelium, Korpal et al. (2008) demonstrated that within 24 h of TGFβ treatment, epithelial architecture is lost and cells begin adopting a mesenchymal phenotype. Significantly, during EMT E-cadherin downregulation was accompanied by suppression of the miR-200 family of miRNA. Ectopic expression of miR-200 family members was sufficient to induce expression of E-cadherin and halt TGFβ-induced EMT, suggesting that they play an important role in promoting epithelial differentiation.

Normal human mammary epithelial cells can also be made to undergo EMT in response to TGFβ as evidenced by the switch from E-cadherin to N-cadherin expression and the adoption of mesenchymal morphological traits. In this way, Eades et al. (2011) demonstrated that the miR-200 family has the most significant deregulated miRNAs during EMT (−1541-fold to −3104-fold) and that miR-200 family gene suppression is mirrored, in vitro by up-regulation of the class III histone deacetylase silent information regulator I (SIRT1). SIRT1, a proposed oncogene in breast cancer is involved in tumour suppressor gene silencing, including the E-cadherin gene CDH1 (O’Hagan et al., 2008). MiR-200a targets a single complimentary binding site in the SIRT1 mRNA 3’ UTR, and the miR-200a promoter region is itself a target for SIRT1 transcriptional repression. It appears, therefore, that miR-200a and SIRT1 are involved in a negative feedback loop that has potentially important downstream consequences for E-cadherin expression and EMT activation (Eades et al., 2011).

ZEB, SNAIL and TWIST: master regulators of EMT under miRNA control

Amongst the most powerful downstream mediators of TGFβ-dependent EMT activation are the Zinc Finger E-box-binding homeobox (ZEB 1 and 2) transcription factors that act in part by repressing the transcription of E-cadherin (Vandewalle et al., 2009). ZEB promotes metastases in a mouse xenograft model, and has been implicated in the progression of several human cancers including breast and colorectal cancer (Spaderna et al., 2006, 2008).

Studies of EMT activation have demonstrated a crucial functional link between the miR-200 family of miRNAs and the ZEB family of transcription factors (Burk et al., 2008; Gregory et al., 2008; Gibbons et al., 2009; Brabletz and Brabletz, 2010). The miR-200 family consists of five members arranged as two clusters, 200a/200b/429 and 200c/141 (Korpal et al., 2008). Forced over-expression of miR-200c in cell models promotes an epithelial phenotype, leads to upregulation of E-cadherin (Hurteau et al., 2007; Korpal et al., 2008) and is sufficient to prevent TGFβ-dependent EMT induction. Furthermore, over-expression in mesenchymal cells can induce MET (Gregory et al., 2008). MiR-200 directly targets the 3’ UTR of ZEB mRNA, downregulates ZEB expression and promotes epithelial differentiation (Hurteau et al., 2007; Gregory et al., 2008; Korpal et al., 2008). Importantly, however, the common miR-200 family promoter region also contains highly conserved ZEB-binding sites through which ZEB 1 and 2 exert reciprocal control at the level of miR-200 transcription (Bracken et al., 2008; Burk et al., 2008). Based on this evidence, it has been postulated that miR-200 and the ZEB family of transcription factors are involved in a double negative feedback loop which acts to stabilize cellular differentiation in response to prevailing extracellular cues, such that strong induction of miR-200 will both promote epithelial differentiation and suppress mesenchymal-specific gene expression by downregulating ZEB 1 and 2 and conversely, ZEB 1 and 2 expression will induce a mesenchymal pattern of gene expression while simultaneously suppressing miR-200 (Brabletz and Brabletz, 2010).

The implications of this proposed feedback loop are potentially profound and may help explain a central dilemma in our understanding of the metastatic
cascade: MiR-200 expression is lost in invasive breast cancer cell lines that convey a mesenchymal phenotype (Gregory et al., 2008) and yet MiR-200 expression is also associated with increased metastatic potential and suppression of Sec23a, a regulator of metastasis-suppressive proteins (Dykxhoorn et al., 2009; Korpah et al., 2011). This apparent paradox helps to illustrate that mechanistically, in order for malignant cells to metastasize, they must be capable of expressing both mesenchymal and epithelial characteristics; the capacity for enhanced motility, invasiveness and resistance to apoptosis required for dissemination and the capacity for colonization and proliferation in order to establish histologically identifiable metastases at distant locations. The ZEB/miR-200 feedback loop may be the mechanism through which this reversible phenotypic switch is achieved, implying that on a molecular level the metastatic programme is dynamic and that tumour cell plasticity in the face of changing environmental cues is a prerequisite for tumour progression (Brabletz and Brabletz, 2010).

This paradigm also links EMT with the concept of cancer stem cells; de-differentiated cells derived from primary tumours that have acquired the capacity for self-renewal and are capable of dissemination and formation of micrometastasis. Mani et al. (2008), using mammary epithelium, demonstrated that EMT activation by ectopic SNAIL or TWIST transcription factor expression produces cells with human breast cancer stem cell antigenic signatures (specifically high CD44 and low CD24 surface expression) and behavioural properties, and furthermore, that naturally occurring neoplastic human mammary stem-like cells co-express markers of EMT. This important observation supports the view that early in the metastatic programme, a sub-population of malignant epithelial cells undertake a de-differentiation step that confers the capacity for both self-renewal and disassociation from their sister epithelial cells. MiRNA profiling of hepatoblastomas—embryonic neoplasms derived from liver progenitor cells—revealed a pattern of miRNA expression resembling that of embryonic stem cells, including over-expression of the miR-371-3 cluster, which has been shown to play a critical role in stem cell renewal (Wang et al., 2008; Cairo et al., 2010).

There is also evidence to suggest that the ZEB/miR-200 feedback loop plays a role in promoting stemness: Shimono et al. (2009), demonstrated that stem-like cells from both normal and malignant mammary tissue under-express miR-200, resulting in the upregulation of the Stem-cell factor BMI-1 and that over-expression of miR-200c reduces both their clonogenic and tumour initiation capacity (Figure 2).

A deeper understanding of the mechanistic link between EMT and stemness remains elusive. However, recent miRNA profiling studies have revealed a number of important insights. The let-7 family of miRNAs control the expression of multiple stem cell characteristics and are markedly downregulated in breast cancer stem cells (Yu et al., 2007). Furthermore, the maturation of primary Let-7 miRNA transcripts is blocked by the stem cell marker Lin28B (Viswanathan et al., 2008). Recently, in cultured PC3 prostate cancer cells, which have undergone EMT in response to PDGF treatment, miR-200b and c have been shown to target Lin28B such that downregulation of miR-200 during EMT is matched by a reciprocal rise in Lin28B expression (Figure 2).

In contrast, Lin28B knockdown using siRNAs leads to strong let-7 induction that in turn, inhibits the capacity for self-renewal of prostate cancer cells. Together, these data provide evidence for the first time, of a possible molecular mechanism through
which EMT and the stem cell phenotype may be co-
induced (Kong et al., 2010).

An upstream role for p53 in this process has also
been described. Loss of p53 function has profound
oncogenic consequences reflecting its pleotropic reg-
ulatory activity in fundamental cellular programs
including cell cycle progression, apoptosis and DNA
repair ( Vousden and Prives, 2009). Several groups
have shown that ectopic p53 expression leads to up-
regulation of numerous miRNAs including miR-200
and miR-192 family members (Chang et al., 2011;
Kim et al., 2011). Chang et al. further demonstrated
that miR-200c is a direct transcriptional target of
p53 (Figure 2) and that in mammary epithelial tissue
p53-dependent miR-200c induction is mirrored by
suppression of important EMT and stem cell markers
including BMI-1 and ZEB. Inhibition of wild-type
(WT) p53 expression in MCF12A, ‘normal’ epithelial
breast cells using siRNA or TGFβ treatment
promotes EMT and a stem-like phenotype, a pro-
cess that can be reversed by ectopic expression of
miR-200c (Chang et al., 2011).

A crucial relationship has therefore been estab-
lished between the loss of p53 function, one of the
most important genetic alterations in human cancer,
EMT, and the concept of cancer stem cells (Schubert
and Brabletz, 2011).

TWIST and SNAIL are the other key EMT-
inducing transcription factors with important roles
in promoting epithelial motility, invasiveness and
metastasis (Cano et al., 2000; Yang et al., 2004).
TWIST directly induces miR-10b, a miRNA highly
expressed in metastatic breast cancer cells compared
with normal mammary tissue. MiR-10b imparts a
pro-invasive, pro-metastatic influence by suppressing
HOXD10 protein expression that in turn induces a
pro-metastatic spectrum of gene expression (Ma
et al., 2007). In vitro, ectopic expression of TWIST in
immortalized, non-tumourogenic HMEC cells leads
to a 4.5-fold increase in miR-10b expression, how-
ever miR-10b alone is not sufficient to induce EMT.
Ectopic expression of miR-10b in non-metastatic
SUM149 and SUM159 human breast cancer cell lines
does, however, induce aggressive invasion and mi-
crometastasis formation in SCID mouse models, pro-
viding experimental validation that over-expression
of individual miRNAs can contribute to metastasis
formation in vivo. However, this is likely to be a re-
fection of HOXD10 suppression, known to enhance
ECM remodelling and cellular mobility, rather than
activation of the EMT programme proper (Ma et al.,
2007).

Vetter and colleagues studied modulations in
miRNA expression during the course of SNAIL-
induced EMT and demonstrated a strong, time-
dependent, upregulation of miR-661, which corre-
lated inversely with the expression of many of its
predicted mRNA targets. Functional studies suggest
that miR-661 is required for efficient breast cancer
cell invasion in vitro, but forced over-expression does
not correspond to a significant increase in invasive
potential. Although miR-661 directly targets Nectin-1,
which has an important role regulating cell polarity
and intracellular adhesion, miR-661-dependent
Nectin-1 suppression is insufficient to induce EMT,
suggesting that other factors are also important in
this process (Vetter et al., 2010).

In an interesting recent development, miRNAs
have also been implicated in anticancer drug re-
sistance mediated by EMT. Drug-induced EMT in
a sub-population of malignant cells may be an
inadvertent consequence of chemotherapy; in ef-
effect, the metastatic cascade being triggered by an
EMT response induced by chemotherapy (Hiscox
et al., 2006; Kajiyama et al., 2007). MiR-448 is
the most downregulated miRNA in chemotherapy-
treated breast cancer cells. Experimental miR-448
suppression induces EMT by promoting SATB1 ex-
pression (AT-rich sequence binding protein1) leading
to increased EGFR-mediated expression of TWIST.
MiR-448 is itself transcriptionally suppressed by NF-
kB with which it engages in a positive feedback me-
chanism mediated by the EGFR/PI3k/AKT signalling
pathway (Li et al., 2011). These findings not only im-
ply that the loss of miR-448 expression in response
to chemotherapy may sow the seed of future recur-
rence, they also reveal a pro-metastatic mechanism,
which having been triggered iatrogenically, may be
a suitable target for drugs aimed at improving the
efficiency of chemotherapy.

MicroRNAs: effectors of change
during EMT

MiRNAs also directly regulate the expression of cell
surface molecules such as E-cadherin, which are im-
portant in defining the differentiation status along
the EMT/MET axis. E-cadherin is a transmembrane
Role of miRNAs in EMT

MiRNA-9 is one miRNA that has recently been found to promote breast cancer progression and target CDH1. MiR-9 is over-expressed in primary breast tumours of patients with metastatic disease compared to metastasis-free patients. Ectopic expression of miR-9 induces an EMT phenotype with a 70% reduction in E-cadherin expression and a reciprocal increase in the expression of vimentin, while inhibition of miR-9 using a miRNA ‘sponge’ suppresses metastasis formation in animal models. Additionally, as a further consequence of E-cadherin downregulation, miR-9 enhances signalling cascades by releasing beta-catenin, normally sequestered at the cytoplasmic membrane by E-cadherin, making it available for nuclear translocation and interaction with the TCF/LEF family of transcription factors and initiating transcription of their oncogenic target genes (Ma et al., 2010).

MiR-137 influences E-cadherin expression indirectly. It has been implicated in melanoma formation (Bemis et al., 2008) and targets the 3′ UTR of Carboxyl-terminal binding protein I (CtBPI), a transcriptional repressor of E-cadherin (Chinnadurai, 2009; Deng et al., 2011). CtBPI and miR-137 expression is inversely correlated in melanoma cell lines and ectopic miR-137 leads to significant E-cadherin upregulation (Deng et al., 2011).

MiR-194 is a further miRNA implicated in regulation of EMT/MET pathways. MiR-194 is highly expressed in normal gastrointestinal and liver epithelial cells, and over-expression of miR-194 in mesenchymal-like cancer cells suppresses migration, invasion and metastasis formation. MiR-194 directly targets the 3′ UTR of several genes including the mesenchymal marker N-cadherin, the small GTP binding protein RAC1, Heparin binding EGF and type I insulin like growth factor, all of which have been implicated in EMT (Meng et al., 2010).

Although over-expression of these and many other miRNAs has been associated with cancer pathogenesis and disease progression, it is important to note that overall, miRNA expression is reduced in cancer (Kumar et al., 2007). Indeed, global miRNA reduction may have a causal role in the onset of the malignant phenotype and recent data have emerged, suggesting that EMT may be a key downstream mechanism through which this is achieved. The cytoplasmic endonuclease Dicer generates mature 18–25 nucleotide miRNAs from pre-miRNA transcripts exported from the nucleus. Dicer is an essential housekeeping gene, the loss of which during knockout experiments proves fatal (Bernstein et al., 2003) and yet for such an important gene it has an unusually long 3′ UTR rendering it susceptible to miRNA control (Martello et al., 2010). This recognition led to the identification of a miRNA family, consisting of miR-103.1, 103.2 and 107, capable of interacting with eight evolutionarily conserved binding sites within the Dicer 3′ UTR, leading to attenuated Dicer expression and global miRNA suppression. MiR-103/107 expression is associated with enhanced metastasis and poor outcome in breast cancer patients and importantly, not only is exogenous miR-103/107 expression sufficient to induce EMT in vitro, it also fosters pro-invasive and pro-metastatic characteristics in mouse mammary metastasis models (Martello et al., 2010).

As discussed, the ZEB/miR-200 feedback loop is a central regulatory mechanism involved not only in controlling transitions along the EMT/MET axis (Brabletz and Brabletz, 2010) but also between highly differentiated and stem-like phenotypes (Chang et al., 2011). The Notch pathway is the most recent genetic program to be identified downstream of ZEB/miR-200 control (Brabletz et al., 2011). Notch signalling is involved in cellular processes including stemness and EMT and aberrant Notch activation has been implicated in a wide range of human cancers (Koch and Radtke, 2007; Rizzo et al., 2008; Wang et al., 2010; Brabletz et al., 2011). MiR-200 family members target Notch pathway components such as Jagged1 (Jag1) and Mastermind-like co-activators, Maml2 and Maml3 (Brabletz et al., 2011). Furthermore, Notch signalling stimulates ZEB1 expression (Wang et al., 2009). In effect, this implies that ZEB-dependent downregulation of miR-200 feeds back positively on ZEB expression and leads to the stabilization of a mesenchymal, stem-like cancer cell phenotype (Brabletz et al., 2011).
Future questions
MiRNAs have powerful roles in the signalling pathways that orchestrate the transition of tumour cells between epithelial and mesenchymal differentiation states, as well as the downstream events through which this plasticity is mediated. They are also key players in carcinogenesis and disease progression pathways and yet surprisingly little data have emerged characterizing genetic or epigenetic defects in miRNA genes that might set these events in motion. The question of whether de novo mutations in miRNA genes trigger EMT remains important, not only because the key to preventing metastasis lies in a deeper understanding of the molecular mechanisms underlying these events, but also because frequently in studies of cancer progression, a causal role for individually deregulated miRNAs is not adequately sought.

A further theme in recent years has been the critical role of the cancer microenvironment and the dynamic interactions between cancer and stromal cells in promoting invasion and disease progression. Studies have shown that deregulated miRNA expression in stromal myofibroblasts provide clinically relevant prognostic information in stage II colorectal cancer, (Nielsen et al., 2011) and it has emerged that miR-21, a critically important oncomiR with pleiotropic actions in a number of tumour scenarios also appears to play a role in TGFβ-dependent myofibroblast transdifferentiation (Yao et al., 2011). As TGFβ drives EMT in the malignant epithelium and the transdifferentiation of myofibroblasts in the stroma and miRNAs are crucial in both events, a greater understanding of the roles of deregulated miRNAs in the interplay between malignant stroma and epithelium becomes an attractive prospect.

Conclusion
EMT and the reverse process MET are complex, multi-faceted and tightly regulated developmental programs that play key roles during morphogenesis, but that become usurped during the process of cancer cell metastasis. As the molecular machinery of EMT/MET has become increasingly transparent, the importance of miRNAs in these processes has become evident, and exposed a further layer of complexity in their regulation. Unravelling miRNA-mediated effects on EMT/MET, and their upstream and downstream targets is likely to uncover novel biomarkers for advanced cancer stages, improve prognostication and reveal new opportunities for therapeutic intervention.

References
Bartel, D.P. (2009) MicroRNAs: target recognition and regulatory functions. Cell 136, 215–233
Battle, E., Sancho, E., Frangi, C., Dominguez, D., Monfar, M., Baulida, J., Monfar, M., Baulida, J. and Garcia De Herreros, A. (2000) The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. Nat. Cell Biol. 2, 84–89
Barna, L.T., Chen, R., Amato, C.M., Classen, E.H., Robinson, S.E., Coffey, D.G., Erickson, P.F., Sheltman, Y.G. and Robinson, W.A. (2008) MicroRNA-137 targets microphthalmia-associated transcription factor in melanoma cell lines. Cancer Res. 68, 1362–1368
Bernstein, E., Kim, S.Y., Carmell, M.A., Murchinson, E.P., Alcorn, H. and Li, M.Z. (2003) Dicer is essential for mouse development. Nat. Genet. 35, 215–217
Brabletz, S. and Brabletz, T. (2010) The ZEB/miR-200 feedback loop—a motor of cellular plasticity in development and cancer? EMBO Rep. 11, 670–677
Brabletz, S., Bajdkak, K., Meidhof, S., Burk, U., Niedermann, G., Dimmler, A., Faller, G., Schubert, J. and Brabletz, T. (2011) The ZEB1/miR-200 feedback loop controls Notch signalling in cancer cells. EMBO J. 30, 770–782
Bracken, C.P., Gregory, P.A., Kolesnikoff, N., Bert, A.G., Wang, J., Shannon, M.F. and Goodall, G.J. (2008) A double-negative feedback loop between ZEB1-SIP1 and the microRNA-200 family regulates epithelial-mesenchymal transition. Cancer Res. 68, 7846–7854
Burk, U., Schubert, J., Wellner, U., Schmalhofer, O., Vincan, C., Spaderna, S. and Brabletz, T. (2008) A reciprocal repression between ZEB1 and members of the miR-200 family promotes EMT and invasion in cancer cells. EMBO Rep. 9, 582–589
Cairo, S., Wang, Y., De Reynies, A., Durokre, K., Dahan, J., Redon, M.J., Fabre, M., McClelland, M., Wang, X.W., Crote, C.M. and Buendia, M.A. (2010) Stem cell-like micro RNA signature driven by Myc in aggressive liver cancer. Proc. Natl. Acad. Sci. U.S.A. 107, 20471–20476
Cano, A., Perez-Moreno, M.A., Rodrigo, I., Locascio, A., Blanco, M.J., Del Barrio, M.G., Portillo, F. and Nieto, M.A. (2000) The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. Nat. Cell Biol. 2, 76–83
Chang, C.J., Chao, C.H., Xia, W., Yang, J.Y., Xiong, Y., Li, C.W., Yu, W.H., Rehman, S.K., Hsu, J.L., Lee, H.H., Liu, M., Chen, C.T., Y., D. and Hung, M.C. (2011) p53 regulates epithelial-mesenchymal transition and stem cell properties through modulating microRNAs. Nat. Cell Biol. 13, 317–323
Chinnadurai, G. (2009) The transcriptional co-repressor CtBP: a foe and a friend of multiple tumor suppressors. Cancer Res. 69, 731–734
Coghill, C. and Murray, G.I. (2010) Current and emerging concepts in tumour metastasis. J. Pathol. 222, 1–15
Comijn, J., Berx, G., Vermanse, P., Verschueren, K., van Grunsven, L., Bruyneel, E., Mareel, M., Huybrecoek, D. and van Roy, F. (2001) The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion. Mol. Cell. 7, 1267–1278
Cottonham, C.L., Kaneko, S. and Xu, L. (2010) miR-21 and miR-31 converge on TIA1 to regulate migration and invasion of colon carcinoma cells. J. Biol. Chem. 285, 35293–35302
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Deng, Y., Deng, H., Bi, F., Liu, J., Bemis, L.T., Norris, D., Wang, X.J. and Zhang, Q. (2011) MicroRNA-137 targets carboxyl-terminal binding protein 1 in melanoma cell lines. Int. J. Biol. Sci. 7, 133–137

Dykxhoorn, D.M., Wu, Y., Xie, H., Yu, F., Lal, A., Petrocca, F., Martinvalet, D., Song, E., Lim, B. and Lieberman, J. (2009) miR-200 enhances mouse breast cancer cell colonization to form distant metastases. PLoS One 4, e7181.

Eades, G., Yao, Y., Yang, M., Zhang, Y., Chumsri, S. and Zhou, Q. (2005) Accumulation of miR-155 and BIC RNA in human B cell lymphomas. Proc. Natl. Acad. Sci. U.S.A. 102, 3627–3632

Gebeschuber, C.A., Zatloukal, K. and Martinez, J. (2009) miR-29a suppresses tristetraprolin, which is a regulator of epithelial polarity and metastasis. EMBO Rep. 10, 400–405

Gibbons, D.L., Lin, W., Creighton, C.J., Rizvi, Z.H., Gregory, P.A., Goodall, G.J., Thilaganathan, N., Du, L., Zhang, Y., Pertz, A. and Kurie, J.M. (2009) Contextual extracellular cues promote tumor cell EMT and metastasis by regulating miR-200 family expression. Genes Dev. 23, 2140–2151

Gregory, P.A., Bert, A.G., Paterson, E.L., Barry, S.C., Tsykyn, A., Farshid, G., Vadas, M.A., Kheew-Goodall, Y. and Goodall, G.J. (2008) The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat. Cell Biol. 10, 593–601

Hiscox, S., Jiang, W.G., Obermeier, K., Taylor, K., Morgan, L., Burni, R., Barrow, D. and Nicholson, R.J. (2006) Tamoxifen resistance in MCF7 cells promotes EMT-like behaviour and involves modulation of beta-catenin phosphorylation. Int. J. Cancer 118, 290–301

Hurteau, G.J., Carlson, J.A., Spivack, S.D. and Brock, G.J. (2007) Over-expression of the microRNA hsa-miR-200c leads to reduced expression of transcription factor 8 and increased expression of E-cadherin. Cancer Res. 67, 7972–7976

Iamimachi, Y., Konig, A., Gress, T. and Menke, A. (2007) Collagen type I-induced Smad-interacting protein 1 expression downregulates E-cadherin in pancreatic cancer. Oncogene 26, 2381–2385

Iorio, M.V. and Croce, C.M. (2009) MicroRNAs in cancer: small molecules with a huge impact. J. Clin. Oncol. 27, 5848–5856

Iorio, M.V., Ferracin, M., Liu, C.G., Veronese, A., Spizzo, S., Sabbioni, E., Magri, M., Pedriali, M., Fabbrini, M., Campiglio, S., Menard, J.P., Palazzo, A., Rosenegard, P., Musiani, S., VBIN, I., Nenci, G.A., Calli, P., Querzoli, M., Napoli, M. and Croce, C.M. (2005) MicroRNA::Gene expression deregulation in human breast cancer. Cancer Res. 65, 7065–7070

Iwatsuki, M., Mimori, K., Yokobori, T., Iahi, H., Beppu, T., Nakamori, S., Baba, H. and Mori, M. (2010) Epithelial-mesenchymal transition in cancer development and its clinical significance. Cancer Sci. 101, 293–299

Kajiyama, H., Shibata, K., Terauchi, M., Yamashita, M., Ino, K., Nawa, A. and Kikawasa, F. (2007) Chemoresistance to paclitaxel induces epithelial-mesenchymal transition through beta-catenin-T-cell factor-4-dependent expression of transforming growth factor beta3. Mol. Biol. Cell 19, 4875–4887

Koch, U. and Radke, F. (2007) Notch and cancer: a double edged sword. Cell. Mol. Life. Sci. 64, 2746–2762

Kokudo, T., Suzuki, Y., Yoshimatsu, Y., Yamazaki, T., Watabe, T. and Miyazato, K. (2008) Snail is required for TGFbeta-induced epithelial-mesenchymal transition of embryonic stem cell-derived endothelial cells. J. Cell Sci. 121, 3317–3324

Kong, D., Banerjee, S., Ahmad, A., Li, Y., Wang, Z., Sethi, S. and Sarkar, F.H. (2010) Epithelial to mesenchymal transition is mechanistically linked with stem cell signatures in prostate cancer cells. Plos One 5, e12455.

Kong, W., Yang, H., He, L., Zhao, J.J., Coppola, D., Dalton, W.S. and Cheng, J.Q. (2008) MicroRNA-155 is regulated by the transforming growth factor beta/Smad pathway and contributes to epithelial cell plasticity by targeting Rhob. Mol. Cell. Biol. 28, 6773–6784

Korpali, M., Lee, E.S., Hu, G. and Kang, Y. (2008) The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. J. Biol. Chem. 283, 14910–14914

Korpali, M., Ell, B.J., Bufla, F.M., Ibrahim, T., Blanco, M.A., Cella-Terrassa, T., Mercatali, L., Khan, Z., Goodarzi, H., Hu, Y., Wei, Y., Yu, G., Garcia, B.A., Ragoussis, J., Amadori, D., Harris, A.L. and Kang, Y. (2011) Direct targeting of Sec23a by miR-200s influences cancer cell secretome and promotes metastatic colonization. Nat. Med. 17, 1101–1108

Kumar, M.S.,卢, J., Mercer, K.L., Golub, T.R. and Jacks, J. (2007) Impaired microRNA processing enhances transformation and tumorigenesis. Nat. Genet. 39, 673–677

Li, Q.Q., Chen, Z.Q., Cao, X.X., Xu, J.D., Xu, J.W., Chen, Y.Y., Wang, W.J., Chen, G., Tang, F., Liu, X.P. and Xu, Z.D. (2011) Involvement of NF-kappaB/miR-448 regulatory feedback loop in chemotherapy-induced epithelial-mesenchymal transition of breast cancer cells. Cell Death Differ. 18, 16–25

Ma, L., Teruya-Feldstein, J. and Weinberg, R.A. (2007) Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. Nature 449, 682–688

Ma, L., Young, J., Prabhala, H., Pan, E., Mestdagh, P., Duth, M., Teruya-Feldstein, J., Reinhardt, F., Onder, T.T., Valastyan, S., Westermann, F., Speleman, F., Vandemesple, J. and Weinberg, R.A. (2010) miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. Nat. Cell Biol. 12, 247–256

Mani, S.A., Guo, W., Liao, M.J., Eaton, E.N., Ayyanan, A., Zhou, A.Y., Brooks, M., Reinhardt, F., Zhang, C.C., Shipitsin, M., Campbell, L.L., Polyak, K., Briskin, C., Yang, J. and Weinberg, R.A. (2008) The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133, 704–715

Martello, G., Rosato, A., Ferrari, F., Manfrin, A., Cordensonsi, M. and Dupont, S. (2010) A microRNA targeting Dicer for metastasis control. Cell 141, 1195–1207

Massague, J. (2008) TGFbeta in cancer. Cell 134, 215–230

Medici, D., Hay, E.D. and Olsen, B.R. (2008) Snail and slug promote epithelial-mesenchymal transition through beta-catenin-T-cell factor-4-dependent expression of transforming growth factor-beta3. Mol. Biol. Cell 19, 4875–4887

Meng, Z., Fu, X., Chen, X., Zeng, S., Tian, Y., Jove, R., Xu, R. and Huang, W. (2010) miR-194 is a marker of hepatic epithelial cells and suppresses metastasis of liver cancer cells in mice. Hepatology 52, 2148–2157

Montserrat, N., Gallardo, A., Escuin, D., Catasus, L., Prat, J., Gutierrez-Avigno, F.J., Peiro, G., Barnadas, A. and Lerma, E. (2011) Repression of E-cadherin by SNAIL, ZEB1, and TWIST in invasive ductal carcinomas of the breast: a cooperative effort? Hum. Pathol. 42, 103–110

Nielsen, B.S., Jorgensen, S., Fog, J.U., Sokilde, R., Christensen, I.J., Hansen, U., Brunner, N., Beker, A., Moller, S. and Nielsen, H.J. (2011) High levels of microRNA-21 in the stroma of colorectal cancers predict short disease-free survival in stage II colon cancer patients. Clin. Exp. Metastasis 28, 27–38
O’Hagan, H.M., Mohammad, H.P. and Baylin, S.B. (2008) Double stranded breaks can initiate gene silencing and SIRT-1 dependent onset of DNA methylation in an exogenous promoter CpG island. PLoS Genet. 4, e1000155.

Rizzo, P., Osipo, C., Foreman, K., Golde, T., Osborne, B. and Miele, L. (2008) Rational targeting of Notch signalling in cancer. Oncogene 27, 5124–5131.

Rodriguez, A., Griffiths-Jones, S., Ashurst, J.L. and Bradley, A. (2004) Identification of mammalian microRNA host genes and transcription units. Genome Res. 14, 1902–1910.

Schubert, J. and Brabletz, T. (2011) p53 spreads out further: suppression of EMT and stemness by activating miR-200c expression. Cell Res. 21, 705–707.

Shimono, Y., Zabala, M., Cho, R.W., Lobo, N., Dalerba, P., Qian, D., Diehn, M., Liu, H., Panula, S.P., Chiao, E., Dirbas, F.M., Somlo, G., Pera, R.A., Lao, K. and Clarke, M.F. (2009) Downregulation of miRNA-200c links breast cancer stem cells with normal stem cells. Cell 138, 592–603.

Slaby, O., Svoboda, M., Fabian, P., Smerdova, T., Knofflickova, D., Bednarkova, M., Nenutil, R. and Vyzula, R. (2007) Altered expression of miR-21, miR-31, miR-143 and miR-145 is related to clinicopathologic features of colorectal cancer. Oncology 72, 397–402.

Spaderna, S., Schmalhofer, O., Hlubek, F., Berx, G., Eger, A., Merkel, S., Jung, A., Kirchner, T. and Brabletz, T. (2006) A transient, EMT-linked loss of basement membranes indicates metastasis and poor survival in colorectal cancer. Gastroenterology 131, 830–840.

Spaderna, S., Schmalhofer, O., Wahlbuhl, M., Dimmler, A., Bauer, K., Sultan, A., Hlubek, F., Jung, A., Strand, D., Eger, A., Kirchner, T., Behrens, J. and Brabletz, T. (2008) The transcriptional repressor ZEB1 promotes metastasis and loss of cell polarity in cancer. Cancer Res. 68, 537–544.

Thiery, J.P. (2003) Epithelial-mesenchymal transitions in development and pathologies. Curr. Opin. Cell Biol. 15, 740–746.

Thiery, J.P., Acloque, H., Huang, R.Y. and Nieto, M.A. (2009) Epithelial-mesenchymal transitions in development and disease. Cell 139, 871–890.

Vandewalle, C., Van Roy, F. and Berx, G. (2009) The role of the ZEB family of transcription factors in development and disease. Cell. Mol. Life Sci. 66, 773–787.

Vousden, K.H. and Prives, C. (2009) Blinded by the light: the growing complexity of p53. Cell 137, 413–431.

Wang, Z., Li, Y., Kong, D., Banerjee, S., Ahmad, A., Azmi, A.S., Ali, S., Abbruzzese, L., Gallick, G.E. and Sarkar, F.H. (2009) Acquisition of epithelial-mesenchymal transition phenotype of gemcitabine-resistant pancreatic cancer cells is linked with activation of the Notch signaling pathway. Cancer Res. 69, 2400–2407.

Yang, J., Mani, S.A., Donaher, J.L., Ramaswamy, S., Itzykson, R.A., Come, C., Savagner, P., Gitelman, I., Richardson, A. and Weinberg, R.A. (2004) Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cancer Cell 117, 927–939.

Zavadil, J. and Bottinger, E.P. (2005) TGF-beta and epithelial-to-mesenchymal transitions. Oncogene 24, 5764–5774.

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