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review

The ZEB/miR-200 feedback loop—a motor of cellular plasticity in development and cancer?

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Epithelial-to-mesenchymal transition (EMT) is a fundamental process in development and disease. Zinc-finger enhancer binding (ZEB) transcription factors (ZEB1 and ZEB2) are crucial EMT activators, whereas members of the miR-200 family induce epithelial differentiation. They are reciprocally linked in a feedback loop, each strictly controlling the expression of the other. Now data show that EMT not only confers cellular motility, but also induces stem-cell properties and prevents apoptosis and senescence. Thus the balanced expression of ZEB factors and miR-200 controls all these processes. We therefore propose that the ZEB/miR-200 feedback loop is the molecular motor of cellular plasticity in development and disease, and in particular is a driving force for cancer progression towards metastasis by controlling the state of cancer stem cells.

Keywords: ZEB1; miR-200; EMT; stemness; cancer; metastasis
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See Glossary for abbreviations used in this article.

Introduction

Controlled activation of stemness and cellular motility are of outstanding importance in embryonic development and adult tissue homeostasis. Uncontrolled activation or maintenance of these properties is associated with the pathogenesis of various diseases, in particular cancer. Pure epithelial and mesenchymal phenotypes mark the extreme endpoints of stationary and highly mobile cell types, respectively. Epithelial-to-mesenchymal transition (EMT) is a reversible embryonic programme that allows partial or complete transition between these extreme phenotypes and is essential for embryonic processes such as gastrulation. However, if EMT is aberrantly activated it is a trigger for tumour progression and metastasis (Thiery *et al*, 2009). It is now known that EMT activation is also associated with the maintenance of stem-cell properties (Mani *et al*, 2008). EMT is activated by key signalling pathways, including the TGF-β, Notch and FGF pathways, which converge in the stimulation of EMT activators—a group of transcription factors repressing epithelial gene expression. This group includes members of the Snail, the bHLH and the ZFH families (ZEB1 and ZEB2; Thiery *et al*, 2009).

MicroRNAs (miRNAs) are small non-coding RNAs that can silence their cognate target genes by binding specifically to mRNAs and inhibiting their translation (Bartel, 2004). The key region of the 18–24-nucleotide-long mature miRNAs is the so-called 'seed sequence', which determines target specificity. So far, approximately 800 miRNAs have been identified in humans. They are potent regulators of gene expression and control diverse cellular processes. miRNAs have also been shown to function either as tumour suppressors or as oncogenes by repressing the expression of important cancer-related genes (Esquela-Kerscher & Slack, 2006).

This article highlights a recently described feedback loop between ZEB factors and members of the miR-200 family of miRNAs. New findings indicate that this loop controls not only EMT, but also other crucial cellular processes, such as stemness, senescence and survival, indicating its central role in embryonic development and in the pathogenesis of many diseases, in particular malignant tumour progression.

Molecular basis: the ZEB/miR-200 feedback loop

ZEB factors (ZEB1 and ZEB2, encoded by the *ZFHX1a* and *ZFHX1b* genes) are transcriptional repressors that comprise two widely separated clusters of C_2H_2-type zinc fingers binding to paired CAGGTA/G E-box-like promoter elements. They induce EMT by suppressing the expression of many epithelial genes, including E-cadherin (Vandewalle *et al*, 2009). Their repressive function is exerted through binding to different co-repressors, such as CtBPs, HDACs and BRG1 (Browne *et al*, 2010; Sanchez-Tillo *et al*, 2010). A central activator of ZEB factors is the TGF-β signalling pathway, indicating that they are crucial intracellular mediators of TGF-β-induced EMT. Enforced expression of ZEB factors in epithelial cells results in a rapid EMT associated with a breakdown of cell polarity, loss of cell–cell adhesion and induction of cell motility. Vice versa, a knockdown of ZEB factors in undifferentiated cancer cells induces a mesenchymal-to-epithelial transition (MET). It is no wonder that these potent factors, if aberrantly overexpressed, have central roles in tumour progression. ZEB1 (also called δ-EF1) is a crucial EMT activator in many human cancers, including prostate, colon, breast and pancreatic (Aigner *et al*, 2007; Graham *et al*, 2008; Spaderna *et al*, 2006; Wellner *et al*, 2009), and it suppresses the expression of basement membrane components (Spaderna *et al*, 2006) and cell polarity factors (Aigner *et al*, 2007; Spaderna *et al*, 2008). Moreover, expression

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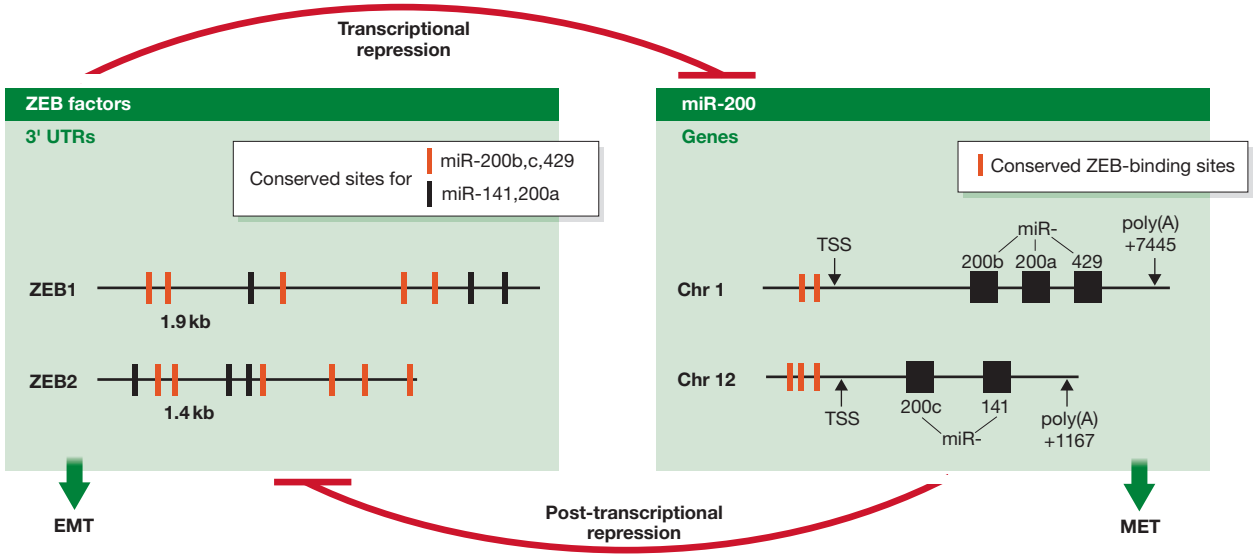

Fig 1 | The ZEB/miR-200 double-negative feedback loop. ZEB factors transcriptionally repress the genes of the miR-200 family members located in two clusters by binding to highly conserved recognition sequences in their promoters. miR-200 family members inhibit expression of ZEB at the post-transcriptional level by binding to highly conserved target sites in their 3′ UTRs. Chr, chromosome; EMT, epithelial-to-mesenchymal transition; MET, mesenchymal-to-epithelial transition; miR, microRNA; TSS, transcriptional start site; UTR, untranslated region; ZEB, zinc-finger enhancer binding.

of ZEB1 promotes metastasis of tumour cells in a mouse xenograft model, indicating a role for ZEB1 in invasion and metastasis of human tumours (Spaderna *et al*, 2008).

The second family member, ZEB2 (also called SIP1), was initially described as a factor collaborating with the TGF-β signalling pathway by interacting with SMAD factors, and was also shown to induce tumour cell invasion (Comijn *et al*, 2001). In contrast to Snail factors, ZEB factors can also interact with transcriptional co-activators, such as p300 and pCAF, and can subsequently switch to a transcriptional activator under still poorly defined conditions (Postigo *et al*, 2003; van Grunsven *et al*, 2006).

miRNAs are known to control central cellular processes, therefore we and others had hypothesized that EMT might also be regulated by miRNAs. Data from several research groups including our own, all independently investigating EMT from different angles, pointed to the involvement of one family of miRNAs, the miR-200 family that includes miR-200a, miR-200b, miR-200c, miR-141 and miR-429 (Burk *et al*, 2008; Christoffersen *et al*, 2007; Gregory *et al*, 2008a; Hurteau *et al*, 2007; Korpal *et al*, 2008; Park *et al*, 2008). By compiling these published data it became clear that members of the miR-200 family can revert an EMT and are powerful inducers of epithelial differentiation. The five miRNAs are located within two clusters on separate chromosomes (Fig 1). They can be further divided into two subgroups according to their seed sequences—subgroup I: miR-141 and miR-200a; subgroup II: miR-200b,c and miR-429—which indicate slight differences in their target gene sets.

How can miR-200 family members exert such a strong EMT-reverting effect? The simple answer lies in their most prominent target factors: all studies so far have described ZEB1 and ZEB2 as the crucial targets of miR-200 family members (Burk *et al*, 2008; Christoffersen *et al*, 2007; Gregory *et al*, 2008a; Hurteau *et al*, 2007; Korpal *et al*, 2008; Park *et al*, 2008). The ZEB1 3′ UTR contains eight miR-200 binding sites (five for subgroup II members and three for subgroup I members), and the ZEB2 3′ UTR contains nine binding sites (six for subgroup II members and three for subgroup I members; Fig 1).

Notably, in addition to the inhibitory effect of miR-200 on ZEB1, we also found a reverse interrelation. Knockdown of ZEB1 led to an increase in the expression of all miR-200 family members (Burk *et al*, 2008). We can demonstrate that ZEB1 directly inhibits transcription of miR-141 and miR-200c genes by binding to highly conserved sites in their common promoter (Fig 1). This finding was corroborated and extended by showing that all miR-200 members are transcriptional targets of ZEB1 and ZEB2 (Bracken *et al*, 2008). These data indicate that ZEB factors and miR-200 family members not only have opposite functions, but also reciprocally control the expression of each other. We have therefore proposed a close functional link between both factor groups in a double-negative feedback loop: the ZEB/miR-200 feedback loop (Burk *et al*, 2008; Fig 1). The consequence of such a reciprocal loop is that the activation of one group of factors would strongly affect the expression and effects of the other group. As ZEB factors are strong EMT inducers, the consequence of miR-200 overexpression is the reduced expression of ZEB factors and subsequent epithelial differentiation. Depending on the extracellular signals, such a loop could easily switch from one to the other side and stabilize either an epithelial or mesenchymal phenotype.

Effects: control of central cellular processes

New data indicate that ZEB factors and miR-200 family members not only regulate EMT, but also control other crucial cellular functions and states, such as stemness—differentiation; longevity—senescence; cell-cycle arrest—proliferation; survival—apoptosis (Fig 2A). Thus the ZEB/miR-200 loop might be a central switch that regulates important intracellular decision processes. We focus on these new aspects of ZEB factors and miR-200 family members, as their roles

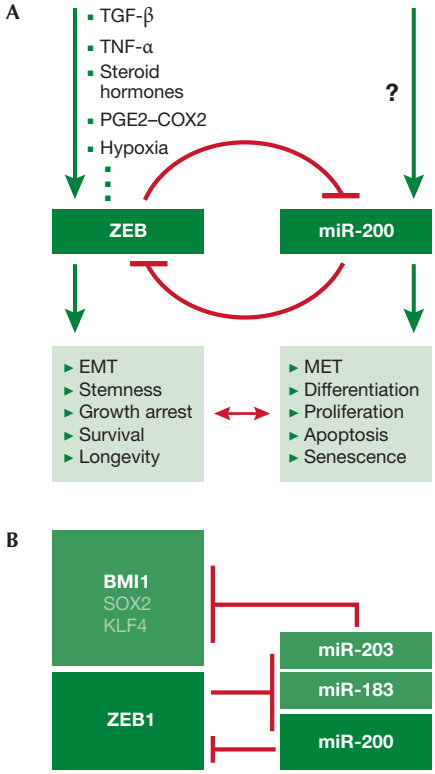

Fig 2 | Processes controlled by the ZEB/miR-200 feedback loop. (**A**) ZEB factors are induced by the indicated factors resulting in activation of the listed processes. Owing to the feedback loop, miR-200 members induce opposite processes. Little is known about factors that activate the expression of miR-200 family members. (**B**) An example showing that ZEB and miR-200 not only block expression of each other, but also ZEB1 suppresses transcription of miR-203 and miR-183, which together with miR-200 inhibit expression of stem-cell factors, linking the induction of EMT and the maintenance of stemness (Wellner *et al*, 2009). EMT, epithelial-to-mesenchymal transition; MET, mesenchymal-to-epithelial transition; miR, microRNA; TGF-β, transforming growth factor beta; TNF-α, tissue necrosis factor alpha; ZEB, zinc-finger enhancer binding.

in controlling typical EMT-associated properties, such as loss of cell–cell adhesion and gain of cellular motility, are already extensively described (Gregory *et al*, 2008b; Peinado *et al*, 2007; Vandewalle *et al*, 2009).

Stemness: differentiation

Is the transition or partial transition from an epithelial to a mesenchymal phenotype molecularly linked to a dedifferentiation programme? If so, this could indicate that certain mesenchymal properties are related to a stemness phenotype. There is increasing evidence that EMT activators and miRNAs also regulate embryonic development and have a role in controlling stemness in both normal stem cells and cancer stem cells (Peter, 2009). We previously postulated a potential link between EMT activation and stemness maintenance in invading tumour cells (Brabletz *et al*, 2005b). Such a link was described at the molecular level, showing that the EMT activator Twist can induce stem-cell properties in mammary epithelial cells (Mani *et al*, 2008; Morel *et al*, 2008). We have recently shown that

Glossary

bHLH	basic helix–loop–helix
BRG1	BRM/SW12-related genes
CDK	cyclin-dependent kinase
CtBP	C-terminal binding protein
EGFR	epithelial growth factor receptor
EMT	epithelial-to-mesenchymal transition
ES cell	embryonic stem cell
FAP1	FAS-associated phosphatase 1
FGF	fibroblast growth factor
HDAC	histone deacetylase
iPS	induced pluripotent stem cells
MET	mesenchymal-to-epithelial transition
miR/miRNA	microRNA
NSCLC	non-small-cell lung cancer
PARP	poly(ADP-ribose) polymerase 1
pCAF	p300/CBP-associated factor
PI3K	phosphatidylinositol-3 kinase
shRNA	short hairpin RNA
TGF-β	transforming growth factor beta
USH	(FOG2) Friend of GATA2
UTR	untranslated region of the mRNA
ZEB	zinc-finger enhancer binding protein
ZFH	zinc-finger homeodomain

ZEB1 increases stem-cell properties and the tumorigenic capacity in pancreatic cancer cells (Wellner *et al*, 2009), and ES cells have been shown to express ZEB1 (Ben-Porath *et al*, 2008), which is reduced on their differentiation. Moreover, ES-cell differentiation is associated with the increased expression of miR-200 family members (Bar *et al*, 2008; Wellner *et al*, 2009), whereas Shimono *et al* (2009) showed that both normal mammary stem cells and breast cancer stem cells have reduced expression of miR-200 family members, which results in increased expression of the stem-cell factor and oncoprotein BMI1. Moreover, the overexpression of miR-200c in normal stem cells or cancer stem cells reduces their clonogenic or tumour-initiating capacities (Shimono *et al*, 2009). We found that ZEB1 not only inhibits the expression of miR-200, but also of miR-183 and miR-203, which together target BMI1 and possibly other stemness-associated factors, such as SOX2 and KLF4 (Wellner *et al*, 2009). Interestingly, some of the stem-cell factors can also induce pluripotent stem (iPS) cells, and further work will show whether the ZEB/miR-200 loop is actually involved in the generation of iPS cells. Finally, the counterpart of miR-200 in *Drosophila*, miR-8, inhibits Wnt signalling—a pathway known to activate the stemness/progenitor cell phenotype in many tissues (Kennell *et al*, 2008). These data are supported by the finding that the Wnt-pathway effector β-catenin is a target of miR-200a (Xia *et al*, 2010). Together these data indicate that EMT and stemness are linked at the molecular level and are controlled by the proposed ZEB/miR-200 loop (Fig 2). Such a miRNA-mediated link between EMT and stemness is probably not a special property of selected tumours, but could be a basal regulatory principle already active in early embryonic and organ development.

Longevity: senescence

Senescence and apoptosis are safeguard programmes that protect cells from neoplastic transformation in response to cellular stress and DNA damage; EMT activators have been shown to be involved in the control of both processes and particularly are able to prevent senescence. For example, Ansieau and co-workers showed

Table 1 | Validated targets of miR-200 family members

Target	miR-200 member	References
ZEB1	All family members	Hurteau *et al*, 2007; Gregory *et al*, 2008a; Park *et al*, 2008; Burk *et al*, 2008; Korpal *et al*, 2008
ZEB2	All family members	Gregory *et al*, 2008a; Park *et al*, 2008; Korpal *et al*, 2008
TGF-β2	miR-141, miR-200c	Burk *et al*, 2008
ERBB receptor feedback inhibitor 1 (ERRFI1)	miR-200c	Adam *et al*, 2009
Friend of GATA 2 (FOG2)	All family members miR-8 (*Drosophila*)	Hyun *et al*, 2009
Polycomb ring finger oncogene (BMI1)	miR-200c	Shimono *et al*, 2009; Wellner *et al*, 2009
WAS protein family member 3 (WASF3, WAVE3)	miR-200b	Sossey-Alaoui *et al*, 2009
β-catenin (CTNNB1)	miR-200a	Xia *et al*, 2010
Class III beta-tubulin (TUBB3)	miR-200c	Cochrane *et al*, 2010
Phospholipase C gamma 1 (PLCG1)	miR-200b/c, miR-429	Uhlmann *et al*, 2010
FAS-associated phosphatase 1 (FAP1)	miR-200c	Schickel *et al*, 2010

miR, microRNA; ZEB, zinc-finger enhancer binding.

that Twist1 can override activated RAS-induced senescence and can cooperate for oncogenic transformation (Ansieau *et al*, 2008). In addition, mouse embryonic fibroblasts from ZEB1-null mice revealed proliferation defects and underwent premature replicative senescence (Liu *et al*, 2008). It was thereby shown that ZEB1 maintains proliferation by transcriptional repression of p21 and $p15^{INK4b}$, two CDK inhibitors taking part in TGF-β signalling. Recently, Ohashi and colleagues also detected that ZEB1 cooperates with mutant p53 to overcome senescence induced by EGFR signalling in oesophageal cancer cells (Ohashi *et al*, 2010). However, in contrast to ZEB1, ZEB2 can induce replicative senescence in cancer cell lines through transcriptional repression of hTERT expression (Lin & Elledge, 2003). Although these data indicate that ZEB1 and ZEB2 might have opposite effects in controlling senescence, it is not clear whether these differences are dependent on the specific cellular background or how they can be explained at the molecular level. As expression of ZEB factors is inhibited by miR-200 family members, these members also indirectly control the senescence state of the cell.

Survival: apoptosis

Controlled apoptosis is important for the homeostasis of tissues with high turnover rates, such as many epithelial tissues. It is also the ultimate mechanism to protect the organism from neoplastic transformation after DNA damage induced by toxic agents. Stem cells have a higher survival capacity than differentiated cells and are more resistant to apoptotic stimuli, as they are the only cells that guarantee tissue reconstitution. It is already known that the induction of EMT is generally associated with reduced apoptosis and increased cell survival. In consequence, cells that have undergone EMT are more resistant to toxic stress, including chemotherapy and radiotherapy (Thiery *et al*, 2009). For example, both Snail1 and Twist1 were shown to confer resistance to cell death and induce drug resistance in cancer cell lines (Li *et al*, 2009a; Vega *et al*, 2004). Similarly, the long-term exposure of breast cancer cells to TGF-β, a highly potent inducer of ZEB1 expression, induces EMT and inhibits apoptosis (Gal *et al*, 2008). We have shown that the shRNA-mediated knockdown of ZEB1 in pancreatic and colorectal cancer cell lines not only affects their stem-cell properties, but also increases the sensitivity of the cells to chemotherapeutic agents such as gemcitabine. Moreover, drug-resistant clones of the gemcitabine-sensitive pancreatic cancer cell line BXPC3 strongly upregulate ZEB1 and reduce expression of miR-200c (Wellner *et al*, 2009). These data are in line with publications by other groups showing that ZEB1 and the associated EMT phenotype confer resistance to chemotherapeutics—such as gemcitabine, 5-FU and cisplatin—in breast and pancreatic cancer cells (Arumugam *et al*, 2009; Shah *et al*, 2007; Wang *et al*, 2009), and that ZEB1 depletion sensitizes NSCLC cells as well as head-and-neck cancer cells to the EGFR inhibitor erlotinib (Buck *et al*, 2007; Haddad *et al*, 2009; Witta *et al*, 2006). Furthermore, expression of ZEB2 protects bladder and squamous cell carcinoma cells from cisplatin- and UV-induced apoptosis by suppressing mitochondrial depolarization and cleavage of PARP and caspase 3 (Sayan *et al*, 2009). Cancer cells are often dependent on certain oncogenic mutations that prevent apoptosis, a phenomenon called 'oncogene addiction'. It was shown recently that pancreatic and lung cancer cells that express ZEB1 can overcome K-RAS-dependent oncogene addiction owing to persisting survival signals after sh-mediated knockdown of oncogenic K-RAS (Singh *et al*, 2009).

The miR-200 side of the feedback loop favours apoptosis and sensitivity to toxic agents. Re-expression of miR-200 family members in breast cancer cell lines restores sensitivity to doxorubicin by increasing the expression of pro-apoptotic genes (Tryndyak *et al*, 2010). Forced expression of miR-200c reverses resistance to chemotherapy in female reproductive cancer cells (Cochrane *et al*, 2010) and to EGFR-mediated therapy in bladder cancer cells (Adam *et al*, 2009). Furthermore, isoflavones, known as natural cancer protective agents, induce the expression of miR-200 family members and subsequently reverse the resistance of pancreatic cancer cells to gemcitabine (Li *et al*, 2009b). Recently, a direct pro-apoptotic function of miR-200c was discovered by showing that this miRNA targets the apoptosis inhibitor FAP1, thereby sensitizing tumour cells to apoptosis (Schickel *et al*, 2010).

Table 2 | Expression of ZEB factors and miR-200 family members in human cancers

Cancer type	Associated features	References
ZEB1		
Lung	Correlates with lack of E-cadherin in NSCLC	Dohadwala *et al*, 2006
	Correlates with resistance to celecoxib and erlotinib in NSCLC	Reckamp *et al*, 2008
Colorectal	Increased levels in cancer cells of invasive regions	Spaderna *et al*, 2006; Aigner *et al*, 2007
Pancreatic	Correlates with poor prognosis	Wellner *et al*, 2009
	Correlates with lack of E-cadherin	Arumugam *et al*, 2009
Gallbladder	Increased levels in invasive tumour cells	Adachi *et al*, 2009
Breast	Increased levels in undifferentiated cancer cells of invasive ductal and lobular cancers	Aigner *et al*, 2007
	Strongly increased levels in triple negative cancers	Graham *et al*, 2009
Ovarian	Increased levels in metastasis	Elloul *et al*, 2010
Endometrial	Increased levels in highly aggressive type II cancers	Spoelstra *et al*, 2006; Singh *et al*, 2008; Hurt *et al*, 2008
Prostate	Correlates with high Gleason score	Graham *et al*, 2008
ZEB2		
Pancreatic	Correlates with lack of E-cadherin	Imamichi *et al*, 2007
Stomach	Increased levels in intestinal type with low E-cadherin	Rosivatz *et al*, 2002
Bladder	Correlates with poor outcome	Sayan *et al*, 2009
Ovarian	Increased levels in effusions	Elloul *et al*, 2005
miR-200		
Pancreatic	miR-200 increased levels in benign PanIN compared with normal duct	du Rieu *et al*, 2010
	miR-200 reduced in pancreatic neuroendocrine tumours	Olson *et al*, 2009
Liver	miR-200c reduced in benign liver tumours	Ladeiro *et al*, 2008
Stomach	miR-141 reduced in gastric cancer	Du *et al*, 2009
Breast	Low expression of miR-200 in basal type compared with other types of breast cancer	Burk *et al*, 2008; Gregory *et al*, 2008a
	Reduced expression of miR-200a/c in metastases compared with primary tumour	Iliopoulos *et al*, 2009
Ovarian	Low expression of miR-200a/b/429 correlates with poor survival	Hu *et al*, 2009
	miR-200 upregulated in ovarian cancer	Nam *et al*, 2008
	High expression of miR-200 correlates with poor prognosis	Bendoraite *et al*, 2010

miR, microRNA; NSCLC, non-small-cell lung cancer; ZEB, zinc-finger enhancer binding.

Growth arrest: proliferation

It is known that the EMT state is often associated with reduced proliferative activity. We made observations that the EMT/stemness phenotype is associated with the growth arrest of invasive tumour cells (Brabletz *et al*, 2001; Jung *et al*, 2001). It became clear that EMT activators directly affect the cell-cycle machinery. It was shown that Snail1 represses cyclin D2 transcription and thereby impairs cell-cycle progression (Vega *et al*, 2004). Moreover, ZEB2 was shown to impair G1–S cell-cycle progression by repressing cyclin D1 transcription (Mejlvang *et al*, 2007). By contrast, recent publications indicate that the switch to an epithelial state (MET) induced by miR-200 members is associated with higher proliferative activity in tumour cells. Overexpression of miR-200 family members increases the proliferation of pancreatic cancer cells (Kent *et al*, 2009) and nasopharynx carcinoma cells (Zhang *et al*, 2010). Interestingly, Uhlmann and co-workers described that only the miR-200b/c/429 cluster, not the miR-141/200a cluster, increases the G2/M population and proliferation in breast cancer cells (Uhlmann *et al*, 2010). These data are corroborated by findings in *Drosophila*. In this case, the miR-200 counterpart miR-8 enhances cell growth and subsequent whole body size by targeting the p85α inhibitor USH/FOG2, thereby leading to activation of the PI3K pathway (Hyun *et al*, 2009). Together, these data show that EMT is often associated with growth arrest. miR-200 members can stimulate proliferation, indicating why re-expression of miR-200 family members and the subsequent MET might be crucial for metastatic colonization.

Consequences: out of control in disease

All the features and processes controlled by the ZEB/miR-200 loop are fundamental cellular processes (a list of miR-200 targets, reflecting the importance of this miR family, is shown in Table 1). In particular, all these properties are important for the regulated maintenance of a stem-cell phenotype. As such, an aberrant and uncontrolled shift

to one side of this loop can have severe consequences for the cell and the organism, and might have an important role in disease pathogenesis. For example, EMT activators such as ZEB1 have been shown to be crucial for the development of organ fibrosis (Kalluri & Weinberg, 2009; Lopez-Novoa & Nieto, 2009). The most relevant disease to the ZEB/miR-200 loop is cancer, in particular in the light of two emerging concepts of cancer initiation and malignant progression. First, on the basis of genetic alterations, aberrant EMT activation is thought to trigger cancer cell motility and thus induce tumour-cell dissemination and metastasis; second, the sustained maintenance of stem-cell properties is the basis of tumour development and progression in the 'cancer stem cell' concept.

On the basis of our own studies of colorectal cancer, we postulated that EMT and stem-cell properties are combined in invasive cancer cells—which often co-express EMT and stem-cell markers—and proposed the 'migrating cancer stem cell' concept (Brabletz *et al*, 2005b). Mani *et al* (2008) showed that EMT and stemness features are indeed linked in breast epithelium and breast cancer cells. The ZEB/miR-200 feedback loop can explain such a link at the molecular level. Switching to the ZEB side of this loop—for example, induction by cytokines such as TGF-β secreted in the invasive tumour environment—can trigger the combined activation of many features crucial for invasion and metastasis of cancer cells. Classical EMT-associated features support tumour cell motility and dissemination, and the inhibition of the apoptotic machinery prevents *anoikis* and allows the cells to survive under the stressful conditions of a new environment. The associated stem-cell properties, including the prevention of senescence, allow unlimited reconstitution of the tumour at a distant site, ultimately leading to metastasis. In addition, there is increasing evidence that these properties are the reason for radiotherapy and chemotherapy resistance, which results in the selection of surviving cancer cells with an EMT and stemness phenotype after therapy.

However, one fact remains to be considered. Most carcinomas have a more or less differentiated phenotype and only few cancer cells, particularly at the invasive fronts, show the EMT phenotype that favours dissemination. Instead, most metastases re-acquire the differentiated state of the primary tumour, indicating that tumour progression is a highly dynamic process based on the plasticity of tumour cells (Brabletz *et al*, 2001, 2005a). Fixation of tumour cells in one phenotypical state could therefore inhibit tumour progression. It has been shown that forced and irreversible overexpression of miR-200b abrogated the metastatic capacity of lung cancer cells (Gibbons *et al*, 2009). However, if the EMT state confers necessary traits to metastasize, why do metastases re-differentiate? Because the EMT/stemness phenotype of tumour cells that favours dissemination is associated with a growth arrest of invasive and disseminating tumour cells (Brabletz *et al*, 2001; Jung *et al*, 2001; Vega *et al*, 2004), such tumour stem cells must consequently switch back to the differentiation and proliferation mode in order to grow. Thus, the switch to the miR-200 side of the loop could be crucial for the growth of both the primary tumour and the metastasis. Recent publications support this view. Expression of miR-200 is indeed associated with an increased metastatic potential of breast cancer cell lines in an isogenic mouse model (Dykxhoorn *et al*, 2009). Moreover, overexpression of miR-200 is associated with increased malignancy of human ovarian cancers (Bendoraite *et al*, 2010).

Thus, a fixation towards neither the ZEB nor the miR-200 sides of the loop alone might favour tumour progression and metastasis.

Sidebar A | In need of answers

(i) What is the relevance of the ZEB/miR-200 feedback loop in normal development?
(ii) What is the individual function and the expression pattern of the five miR-200 family members in normal development and tumours?
(iii) How is expression of miR-200 family members induced?
(iv) What is the specific function of ZEB1, ZEB2 and the other EMT inducers?
(v) Can the direction of the ZEB/miR-200 loop be manipulated by drugs?

Rather, it is the aberrant ability of the tumour cell to use this loop and switch from one side to the other that allows it to adapt to the demanding conditions of the changing environment. We therefore suggest that the ZEB/miR-200 loop is the molecular motor of tumour-cell plasticity and is the crucial prerequisite for cancer progression. This view is fostered more and more by publications showing that, in an increasing number of human cancer types, the expression of ZEB factors and miR-200 family members correlates with their clinical behaviour (Table 2).

Conclusions

New data show that EMT not only confers cellular motility, but also induces stem-cell properties, and prevents apoptosis and senescence. These processes provide the basis for cellular plasticity in development and adult tissue homeostasis. Increasing evidence shows that they are regulated by a balanced expression of ZEB factors and miR-200 family members, which are reciprocally linked in the ZEB/miR-200 feedback loop. We propose that this feedback loop is a molecular motor of cellular plasticity in development and disease, and in particular is a driving force for cancer progression towards metastasis. However, many questions remain to be answered (Sidebar A). The clarification of these points might lead to new therapeutic options for diseases such as fibrosis and cancer.

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