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Cyclin A1 Expression is Reciprocally Controlled by the Transcription Factor *ZNF217* and miRNAs in Invasive Breast and Prostate Cancer Cells: An *In Silico* Analysis

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Abstract

Cyclins and their partner cyclin-dependent kinases (CDKs) play crucial roles in proliferation, initiation of DNA replication, and mitosis. Human cyclin A1 is expressed at its highest levels in spermatocytes and is re-expressed in leukemic cell lines, in cells from acute myeloid leukemia (AML) and acute promyelocytic leukemia (APL), as well as in metastatic breast cancer, hepatocarcinomas and prostate cancer. Transgenic mice engineered to express cyclin A1 in myeloid precursor cells develop AML with low penetrance and long latency. In this work we have mined and analyzed data from the Gene Expression Omnibus (GEO) that potentially reveal novel cyclin A1 mechanisms of action in metastatic breast and prostate tumors. The data suggest that in breast and prostate tumors, cyclin A1 expression is repressed by two miRNAs and activated by the transcription factor *ZNF217,* and that cyclin A1 is overexpressed in invasive prostate cancer but not in pre-invasive carcinoma. Down-regulation of the miRNAs and overexpression of *ZNF217* correlate with epithelial-to-mesenchymal transition (EMT), suggesting that EMT may be partly mediated by ZNF217-mediated re-expression of cyclin A1. Collectively, these data argue that re-expression of cyclin A1 protein may contribute to the mesenchymal-to-epithelial transition in the aforementioned tumor types. Since cyclin A1 protein is not expressed in normal adult tissues, except germ cells, it may be a promising target for intervention. We discuss implications of these findings in further dissecting the role of cyclin A1 protein in cancer development and for its targeting.

Keywords Spermatocytes; Cyclin A1 protein; Breast cancer; Prostate cancer; Acute myeloid leukemia; Acute promyelocytic leukemia

Abbreviations: AML: Acute Myeloid Leukemia; ATRA: All-trans Retinoic Acid; CDK: Cyclin Dependent Kinase; MDM2: Mouse Double Minute; miRNA: microRNA; ZNF217: Zinc Finger Protein 217.

Introduction

Cyclin A1 in human myeloid leukemias

The human cyclin A1 was first identified in the mouse and in human AML cell lines by virtue of its high levels of expression [1-3]. It is expressed in nearly 99% of all AML samples and its mRNA has been detected in several myeloid leukemia cell lines, including ML-1, U937, NB4, KG-1, and THIZ1. Molecular mechanisms underlying the oncogenic activation of cyclin A1 in various types of cancers as well as its role remain unknown. Cyclin A1 is required for normal meiosis in the mouse [4] and its forced overexpression in transgenic mice leads to a myelodysplastic syndrome and eventually to leukemia, although with low penetrance and long latency [5]. In normal hematopoietic cells cyclin A1 is predominantly localized in the nucleus however it is found in the cytoplasm in cells from AML patients, suggesting that its involvement in the transformation of leukemic cells may be linked to forming predominantly alternative complexes with the cyclin dependent kinases, *CDK1* and *CDK2* and with novel substrates, activating their kinase activity [6,7]. It has been shown that cyclin A1 plays a role in mediating the response of leukemic cells to ATRA-treatment. Treatment with ATRA enables the relocalization of cyclin A1 from the cytoplasm to the nucleus [8,9]. Several studies have demonstrated that cyclin A1 is important in DNA repair mechanisms by binding to the nuclear proteins that are required for DNA repair. Altered expression and modification of cyclin A1 protein is associated with sensitivity to apoptosis induced by anti-tumor therapeutic agents [10,11]. These data suggest that cyclin A1 expression plays a role in the etiology of myeloid leukemias and is likely to be a downstream target of the aberrant fusion proteins that characterize APL.

Role of cyclin A1 in solid tumors

The role of cyclin A1 in solid type tumors is less well known. Data exist for breast cancer cell lines warranting further exploration given its tissue-restricted expression. Recently it was shown that cyclin A1 contributes to prostate cancer cell invasion via interactions with the VEGF and AR pathways [12,13]. Cyclin A1 tumorigenic pathways are closely linked with developmental networks such as pathways where the *six1* homeobox protein is active. Oncogenic induction of *six1* causes cyclin A1 levels to increase and correlates with enhanced proliferation of mammary carcinoma cells whereas knockdown of *ccnA1* in the same cells prevents their proliferation when *six1* is ectopically overexpressed.

Role of miRNAs in solid tumors

Metastatic burden can be heavy in cancer. An understanding of molecular mechanisms of metastasis may lead to the identification of suitable targets, especially among the miRNAs some of which have metastasis-promoting or metastasis-inhibiting roles. Several pathways are affected by interactions with miRNAs. Specifically, *miR-335* and *miR-205* are found to be either deleted or under-expressed in breast and prostate cancers with *miR-335* commonly deleted in human breast cancers. Notably, whereas *miR-335* is critical for breast cancer reinitiation [14], *miR-205* is involved in invasive cancer. For example, compared to matched normal prostatic tissues, *miR-205* expression levels are reduced in prostate cancer cell lines as well as in tumor, especially in patients with local-regionally disseminated disease [15].

The restoration of the expression of *miR-205* in prostate cancer cells results in reversal of the mesenchymal-to-epithelial transition. This is indicated by up-regulation of E-cadherin, an epithelial cell marker, and by reduction of cell mobility and invasion. Also, it results in the down-regulation of several oncogenes, such as interleukin 6, caveolin-1 and *EZH2*, which are known to be involved in disease progression. Interestingly, enforced expression of the *miR-200* family alone is sufficient to prevent TGF-beta-induced EMT, the reverse of MET. Ectopic overexpression of *mir-200* microRNAs in mesenchymal cells reverses the mesenchymal to epithelial transition by targeting ZEB. Expression of these microRNAs is lost in invasive breast cancer cell lines with the mesenchymal phenotype. Notably, expression of *miR-200* family members is also lost in metaplastic breast cancer specimens lacking E-cadherin [16].

Role of the zinc-finger, transcription factor *ZNF217* in solid tumors

The Krüppel-like zinc finger protein *ZNF217* has been established as an oncogene in breast cancer and it may play critical roles in other solid tumors. Enhanced expression of *ZNF217* mRNA correlates with poor prognosis and the development of metastases in breast cancer. Moreover, its overexpression in breast cancer cells stimulates migration and invasion *in vitro* and induces lung or node metastases in mice *in vivo*.

Notably, *ZNF217* also induces epithelial-to-mesenchymal transition (EMT) in human mammary epithelial cells, through the TGF-β-activated, SMAD signaling pathway [17]. Also, in lung carcinoma cell lines, *ZNF217* may act as an oncogene by recruiting deacetylase/demethylase complexes by interacting with *MDM2* to inhibit acetylation of *p53* [18].

In this work, we have mined gene expression microarray data which demonstrate the following:

A) *CcnA1* mRNA levels are reduced by expressed *miR-335* and *miR-205* in breast and prostate cancer cells.

B) knock-down of either miRNA with siRNA increases the levels of *ccnA1* mRNA, suggesting a role in its regulation.

C) Cyclin A1 expression levels correlate with invasive prostate cancer

D) That the transcription factor *ZNF217* increases expression of *ccnA1* mRNA, in invasive breast cancer cells.

We discuss plausible mechanisms of control of the epithelial-to-mesenchymal transition by cyclinA1 and ZNF2117.

Materials and Methods

Using the term "*cyclin A1 and invasive and cancer*" as a query in the Gene Expression Omnibus, GEO, database, returned 50 studies of which only three fulfilled the criterion of *ccnA1* mRNA expression levels being significantly changed by re-expression of miRNAs (Table 1). Data were downloaded as SOFT files in Excel and subjected to statistical analysis using Student's t-test in order to evaluate the significance of change in expression levels of the *ccnA1* gene. Gene expression data for *ccnA1* from study GDS 3138 [14,19] were analyzed with Student's t-test for significance.

GEO STUDY	VARIABLE	1st AUTHOR	PMID
GDS3138	*MIR-335*	Tavazoi, Png	18185580, 21289068
GDS3634	*MIR-205*	Gavidline	19244118
GDS4885	*ZNF217*	Vendrell	25593193

Table 1: GEO gene expression studies showing significant changes in *ccnA1* mRNA levels by miRNAs and *ZNF217*.

The null hypothesis was that there is no significant difference between control and experimental samples. Y-axis: arbitrary normalized transcriptomic microarray expression values. Three expression values were used for each group and a p-value was computed for each one.

Results

The epithelial-to-mesenchymal transition (EMT) in invasive breast and prostate cancers is a complex phenomenon [20-22]. It is critical for cancer invasive behavior, and is currently studied with the aim of finding suitable targets [23,24]. In this work we have mined publicly available data from breast and prostate cancer cell lines to identify links between microRNA signatures, the transcriptional factor ZNF2117 and cyclin A1 in invasive breast and prostate cancers. Our rationale was based on the fact that miRNAs suppress cyclin A1 expression levels in these tumors and counteract the activating effect of the transcription factor *ZNF217*. We identified three datasets (Table 1) in GEO that report results of the effect of overexpressing *miR-335* (breast) and *miR-205* (breast and prostate cancer respectively) on the levels of cyclin A1 mRNA. We extracted the data for cyclin A1 and subjected them to elementary statistical analysis in order to validate that significant differences exist in cyclin A1 levels between non-invasive and advanced invasive cancers. Re-expression of *miR-335* in LM2 breast cancer cells reduces the levels of cyclin A1 mRNA by at least 50% (Figure 1A, p=0.0045) and this correlates with a decrease in invasive behavior. In DU145 prostate cancer cells, re-expression of *miR-205* (Figure 1B, p=0.0020) also leads to significantly decreased *ccnA1* mRNA levels. In a study of stromal cells in a mouse model on breast cancer invasiveness (Table 1, GDS2443), the levels of *ccnA1* mRNA were significantly increased in invasive prostate cancer cells (Figure 1C) but were reduced in intraepithelial, non-invasive prostate neoplasia (Figure 1C, p=0.0018). Since the levels of these miRNAs are substantially reduced in these invasive cancers, we conclude that they might act as tumor suppressors and that in their absence, *ccnA1* mRNA levels are increased, contributing to invasive growth. Notably, in both tumor types, the absence of these miRNAs and overexpression of cyclin A1 correlate with metastasis. On the other hand, restoring the expression of these microRNAs in malignant LM2 breast cancer cells suppresses lung and bone metastasis by human cancer cells *in vivo* [14,19].

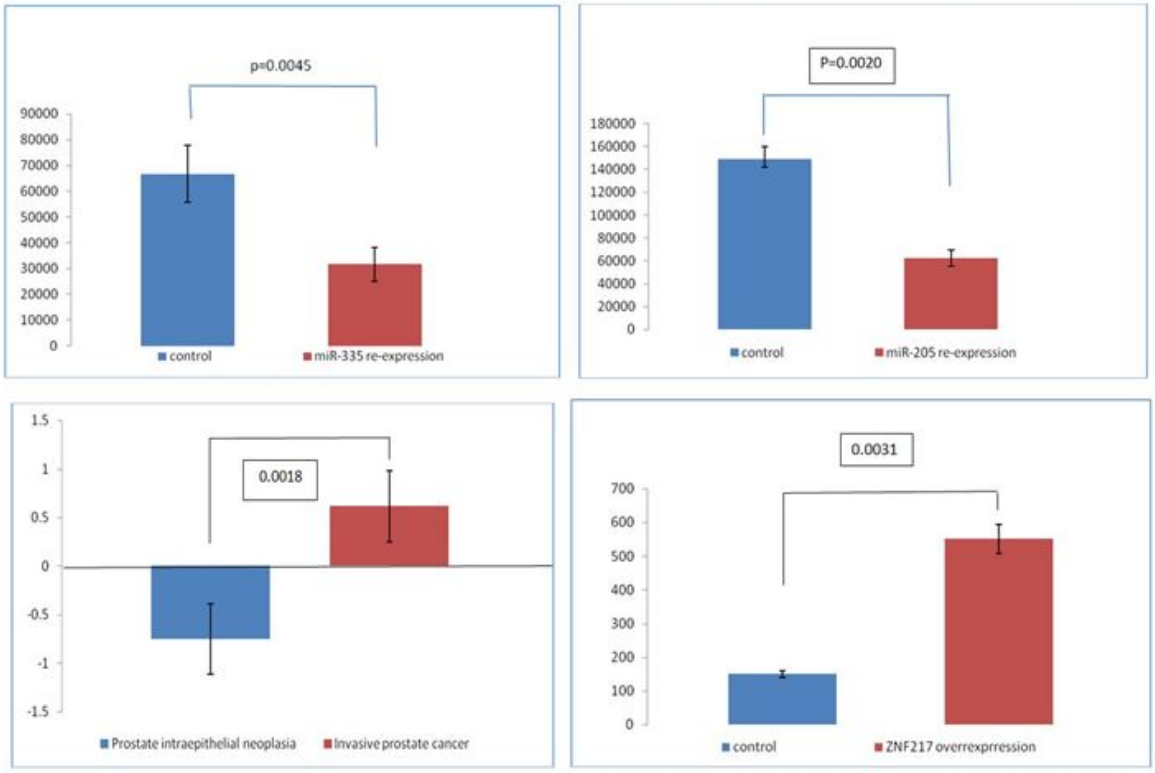

Figure 1: (Upper left panel): Re-expression of *miR-335* in LM2 breast cancer cells suppresses *ccnA1* mRNA expression. (Upper right panel): Re-expression of *miR-205* in LM2 breast cancer cells suppresses *ccnA1* mRNA expression (Lower left panel): Induction of endogenous *ccnA1* mRNA in invasive prostate cancer of mouse stromal cells (red bar) but not in intraepithelial neoplasia (blue bar). Y-axis: log2 ratios of transcriptomics microarray expression data. (Lower right panel): Ectopic overexpression of *ZNF217* from a retrovirus vector in breast cancer cells induces the levels of *ccnA1 mRNA*.

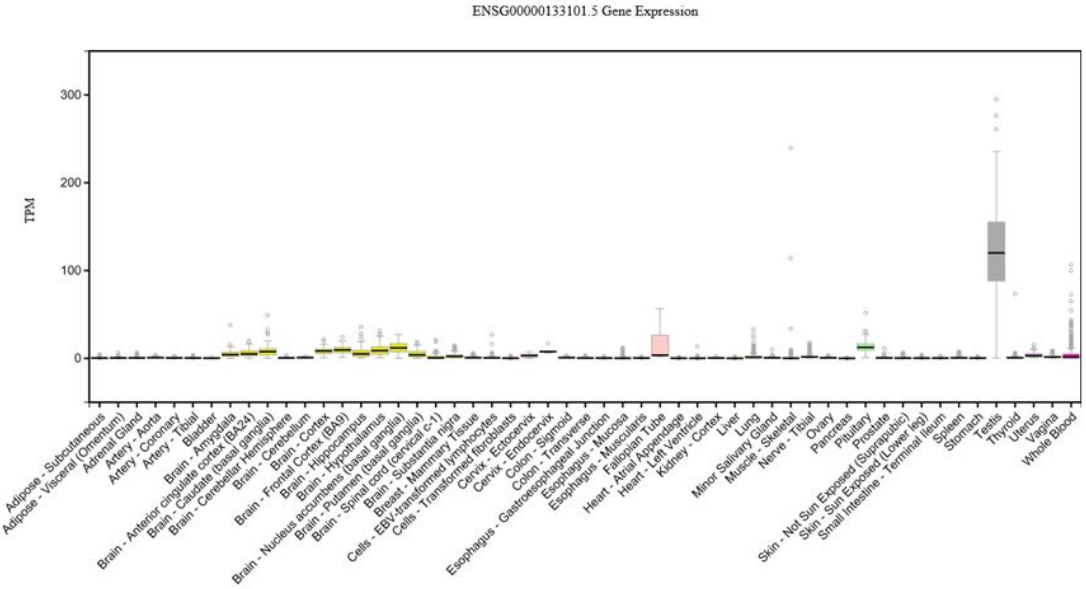

Figure 2: A) *ccnA1*mRNA levels from various tissues, expressed as transcripts-per-million, TPM, were extracted from the GETX portal database (www.gtexportal.org): *ccnA1* mRNA expression is virtually restricted to the testis.

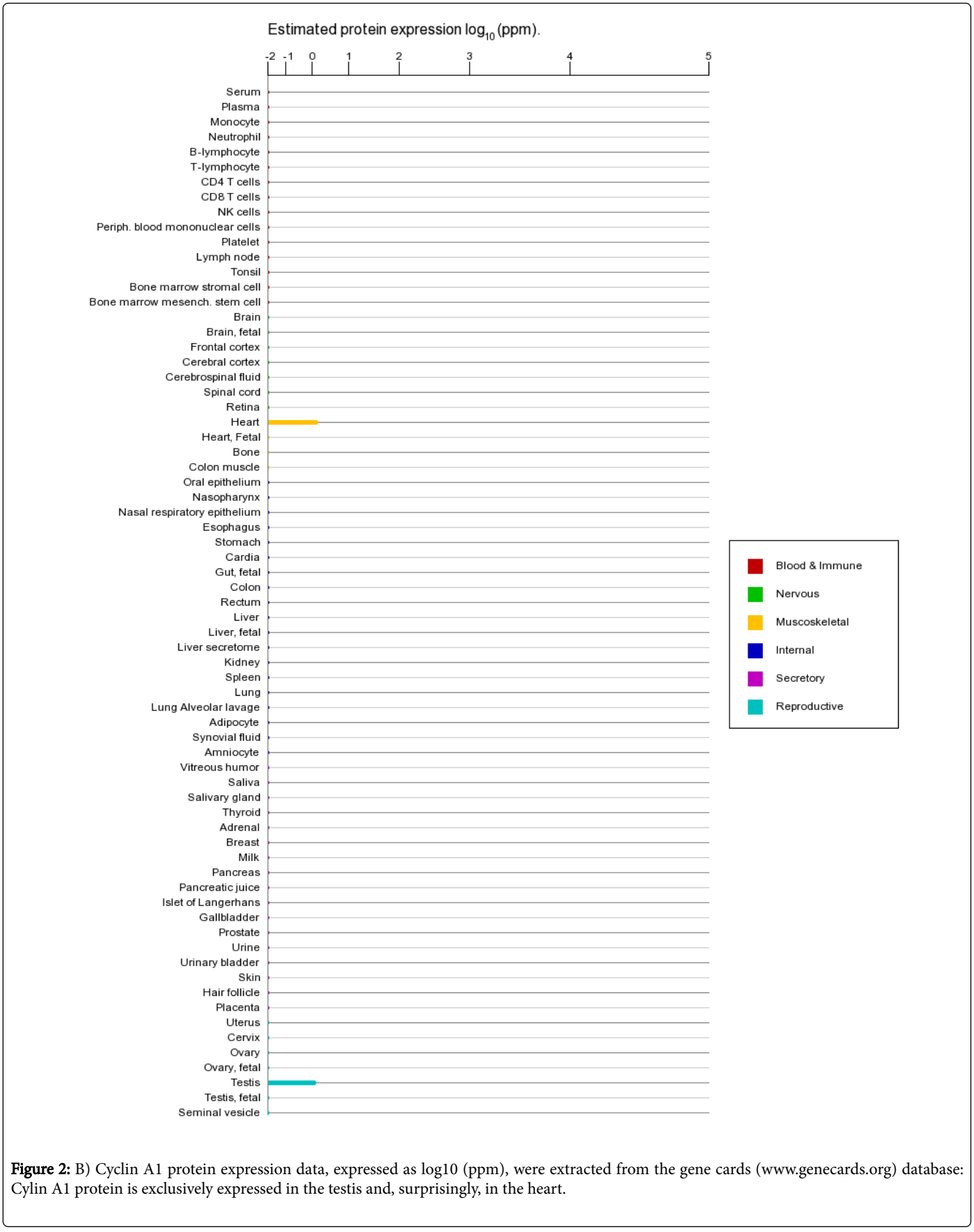

Figure 2: B) Cyclin A1 protein expression data, expressed as log10 (ppm), were extracted from the gene cards (www.genecards.org) database: Cylin A1 protein is exclusively expressed in the testis and, surprisingly, in the heart.

In conclusion, we have identified two miRNAs that suppress *ccnA1* expression in two cancer cell lines with invasive properties and also we have found that overexpression of *ZNF217* correlates with activation of *ccnA1* mRNA expression. When viral constructs of *miR-335* and *miR-205* are introduced into these cells, they reduce *ccnA1* mRNA and protein levels significantly and inhibit invasiveness of LM2 cells. Moreover, *miR-205* not only down-regulates expression of *ccnA1*, but also, it promotes the mesenchymal-to-epithelial transition and reduces tumorigenicity in DU145 prostate cancer cells [17]. In terms of message and protein expression levels in adult cells, *ccnA1* mRNA is expressed predominantly in testis and only at low levels in other tissues (Figure 2A), whereas the protein is exclusively expressed in developing spermatocytes in the testis (Figure 2B). Surprisingly, protein also is found in the heart (Figure 2B), however its significance remains unknown.

Discussion

Expression of *miR-335* is lost in the majority of primary breast tumors from patients who relapse, and the loss of expression of microRNA is associated with poor, distal metastasis-free survival. Therefore, restoring the expression of these microRNAs in malignant LM2 cells could suppress lung and bone metastasis by human cancer cells *in vivo*. In agreement with these findings, re-expression of *miR-335* in breast cancer cell lines leads to loss of invasive ability decreased cyclin A1 mRNA and reversal of EMT. *MiR-335* is thus identified as a metastasis suppressor miRNA in human breast cancer cell lines and *miR-205* in both breast and prostate cells. The correlation of *miR-335* and cyclin A1 expression is indicative of their opposite roles in the biology of breast cancer cells. The data suggest that expression of *miR-335* is part of a network of growth control that counteracts expression programs of growth in which cyclin A1 participates.

In breast or prostate cancer cells, EMT, and its reverse, MET, are linked to invasion and are controlled by, among other factors, the transcription factor *ZNF217*. Overexpression of *ZNF217* in breast cancer cells enhances *in vitro* migration and invasion and *in vivo* it contributes to the development of lung or node metastases in mice. *ZNF217* is capable of promoting EMT in human mammary epithelial cells, and activates the TGF-β-activated, SMAD signaling pathway. We found that in breast cancer cells, overexpression of *ZNF217* in non-metastatic breast cancer cells, leads to cyclin A1 mRNA overexpression and this correlates well with invasive behavior and EMT suggesting that cyclin A1 may be instrumental in promoting EMT by overexpressed *ZNF217* (Figure 1D, p=0.0031).

In both invasive breast and prostate cancer, cyclin A1 might be reciprocally regulated by *mir-335*, *miR-205* and *ZNF217* and this could be relayed to ZEB which is a key factor in controlling EMT (Figure 3). Lower *miR-335/205* expression levels and increased *ZNF217* levels, could act as an additional signal which leads to cyclin A1 overexpression thus forcing cells to transit to an invasive phenotype. Since *ZNF217* induces metastasis through the TGFβ/SMAD pathway ([17,24], we propose that it may do this partly *via* cyclin A1. As cyclin A1 expression is activated by the *six1* oncogene, leading to EMT, this might be an alternative pathway of action by *ZNF217*/cyclin A1. Whether these pathways converge to ZEB, a key factor in EMT and invasion, and whether cyclin A1 acts through ZEB remain to be established (Figure 3). As a corollary, the restoration of *miR-335/205* expression in breast and prostate cancer cells could open an approach for reducing invasiveness. It is known that re-expression of these miRNAs results in cell rearrangements consistent with reversal as indicated by up-regulation of E-cadherin and reduction of cell locomotion and invasion, as well as in the down-regulation of several oncogenes known to be involved in disease progression (i.e., interleukin 6, caveolin-1, *EZH2*). The evidence suggests that these events are driven by the concurrent repression of specific predicted *miR-205* targets, such as N-chimaerin, *ErbB3*, *E2F1*, *E2F5*, *ZEB2*, and protein kinase Cε. In conclusion, we have determined that *miR_335*, *mir-205* and *ZNF217* control the levels of the cyclin A1 mRNA and this may implicate this cyclin in the control of EMT in invasive breast and prostate cancer cells.

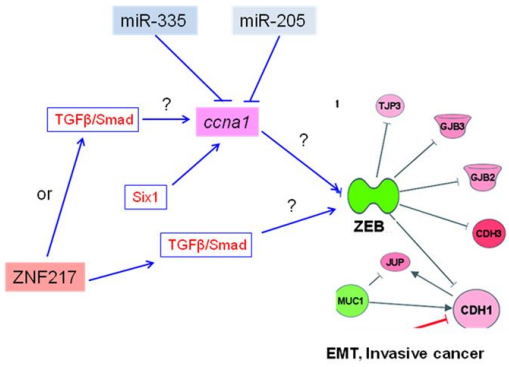

Figure 3: Schematic depiction of possible links of various miRNAs and of *ZNF217* with cyclin A1 in invasive cancer. *miR-335* and *miR-205* suppress expression of cyclin A1 whereas ZNF2117 increases it. Shown are possible routes of action by *ZNF217* on EMT and invasion. Experimental evidence indicates that *ZNF217* promotes EMT and invasion through the TGFβ/SMAD pathway. Another mechanism might be through cyclin A1. Whether all converge to ZEB, a critical factor for EMT/invasion also is unknown. Our analysis formulates new hypotheses that can be experimentally tested.

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Conflict of Interest

The authors declare no conflict of interest of any kind

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