Role of microRNA-203 and microRNA-205 in Lung Cancer Metastasis

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Background and Rationale

- Lung cancer is the first cause of cancer-related deaths in both female and males in the USA (Fig 1).[1] 83% of lung cancers are classified as Non-Small Cell Lung Cancer (NSCLC).[1]
- The 5-year survival rate for metastatic lung cancer is only 4%.[1] Thus, it is imperative that the molecular mechanisms driving metastasis be fully elucidated.
- Epithelial–mesenchymal transition (EMT) is a process through which cells lose their epithelial properties and become invasive and migratory. EMT has been associated with metastasis and chemoresistance (Fig 2).[2,3]
- Micro-RNA's (miRs) are non-coding RNAs that inhibit target genes by degrading their mRNA or inhibiting translation.[4] The miR-200 family has been shown to regulate EMT and metastasis (Fig 3).[5]
- In addition to miR-200, miR-203 and miR-205 were significantly repressed in mesenchymal-like human NSCLC cell lines (Fig 4).[6]

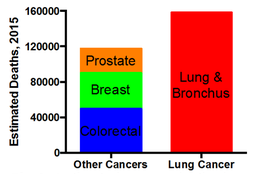


Fig 1. Estimated cancer deaths by site, 2015. ***Modified from Amer Cancer Soc, 2015.***

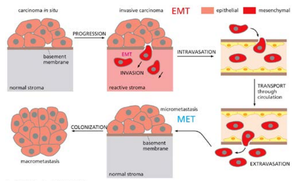


Fig 2. EMT as a model for metastasis. ***Weinberg, The Biology of Cancer, 2014.***

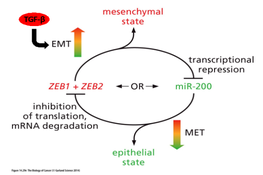


Fig 3. miR-200-Zeb1 axis regulates EMT and metastasis. ***Modified from Weinberg, The Biology of Cancer, 2014.***

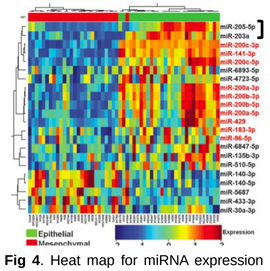
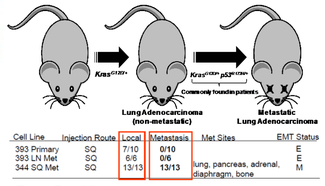


Fig 4. Heat map for miRNA expression profiles of 55 human NSCLC lines, stratified into epithelial and mesenchymal categories based on their EMT score. ***Kundu et al, Oncogene, 2015***



Cell Line	Injection Route	Local	Metastasis	Met Sites	EMT Status
393 Primary	SQ	7/10	0/10		E
393 LN Met	SQ	6/6	0/6		E
344 SQ Met	SQ	13/13	13/13	lung, pancreas, adrenal, diaphragm, bone	M

Fig 5. Model of lung adenocarcinoma metastasis (KP mice). ***Zheng et al, Oncogene, 2007. Gibbons et al, Genes Dev, 2009.***

Hypothesis

Down-regulation of miR-203 and miR-205 in NSCLC promotes tumor migration and invasion.

Results

Fig 6. miR-203 is down-regulated in human and mouse mesenchymal NSCLC cells.

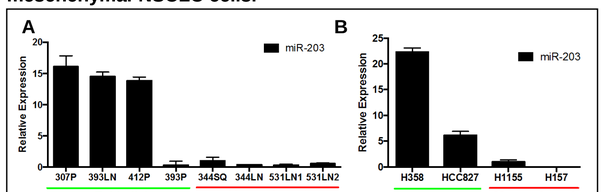


Figure 6. A,B Relative expression of miR-203 and miR-205 in a panel of epithelial-like or mesenchymal-like mouse **(A)** and human **(B)** NSCLC lines.

Fig 7. Effect of restoration of miR-203 and miR-205 in mesenchymal cells.

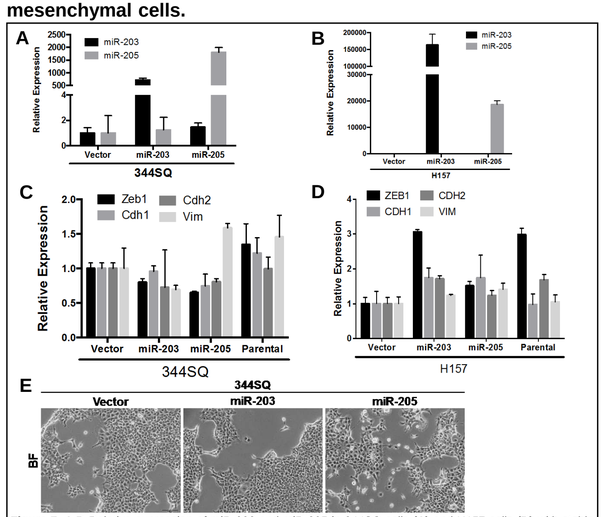


Figure 7. A,B Relative expression of miR-203 and miR-205 in 344SQ cells **(A)** and H157 cells **(B)** with stable constitutive expression of pL-GFP vector, miR-203 or miR-205. **C,D** Relative expression of various EMT markers in 344SQ cells **(C)** and H157 cells **(D)** with stable constitutive expression of pL-GFP vector, miR-203 or miR-205. **E,** Bright field microscopy images showing morphology of 344SQ cells with stable constitutive expression of pL-GFP vector, miR-203 or miR-205.

Fig 8. Effect of miR-203 and miR-205 in cell migration and invasion.

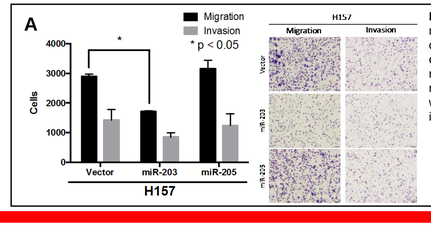


Figure 8. A, Trans-well migration or invasion of H157 cells with stable constitutive expression of pL-GFP vector, miR-203 or miR-205 and representative images of trans-well chambers. (Significance is indicated by *$P \leq 0.05$).

Results (Continued)

Fig 9. Regulation of miR-203 and miR-205 in an experimental EMT model.

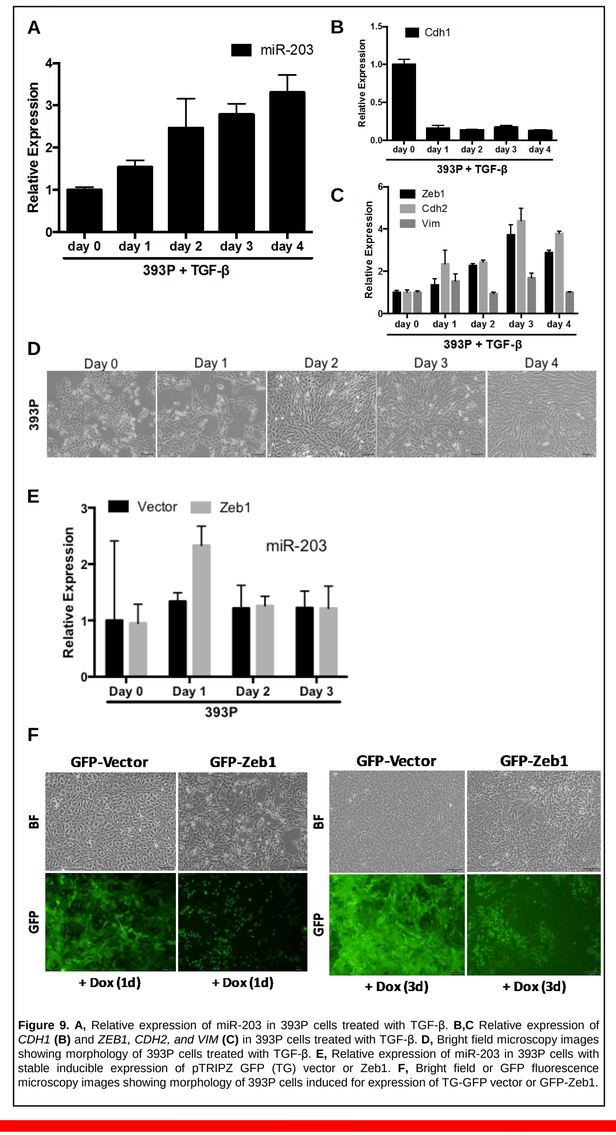


Figure 9. A, Relative expression of miR-203 in 393P cells treated with TGF-β. **B,C** Relative expression of *CDH1* **(B)** and *ZEB1, CDH2, and VIM* **(C)** in 393P cells treated with TGF-β. **D,** Bright field microscopy images showing morphology of 393P cells treated with TGF-β. **E,** Relative expression of miR-203 in 393P cells with stable inducible expression of pTRIPZ GFP (TG) vector or Zeb1. **F,** Bright field or GFP fluorescence microscopy images showing morphology of 393P cells induced for expression of TG-GFP vector or GFP-Zeb1.

Results (Continued)

Figure 10. Screening for miR-203 and miR-205 targets.

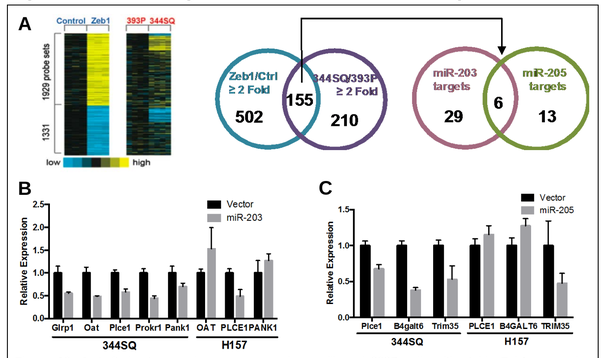


Figure 10. A, Left: heat maps for gene expression profiles of 393P cells with stable Zeb1 expression compared with pCDNA vector control cells (genes represented are differentially expressed ($P < 0.01$, fold change> 2) and metastatic 344SQ cells compared with non-metastatic 393P cells. Right: Venn diagram with the number of genes overlapping for the different data sets as indicated. **B,C** Relative expression of predicted targets of miR-203 **(B)** and miR-205 **(C)** in 344SQ cells and H157 cells with stable constitutive expression of pL-GFP vector, miR-203 or miR-205.

Conclusions

- We validated the down-regulation of miR-203 in mesenchymal cells in a mouse cell line panel and human cell line panel.
- miR-203 and miR-205 overexpression does not alter the expression of EMT markers.
- miR-203 significantly reduced cell migration. Although results were not statistically significant, miR-203 markedly reduced cell invasion.
- miR-203 expression increased upon TGF-B treatment. Zeb1 overexpression does not seem to affect miR-203 expression.
- Predicted targets suggest miR-203 and miR-205 may play a role in regulating cancer metabolism.
- More experiments are needed to further elucidate the role of miR-203 and miR-205 in NSCLC metastasis.

Future Directions

- Co-expression of miR-203 and miR-205
- *In vivo* over-expression of miR-203 and miR-205
- 3'UTR Luciferase assays of predicted target genes
- Anti-miR-203 and Anti-miR-205
- Functional validation of targets (GOF, LOF)

References

1) Amer Cancer Soc, 2015, Cancer Facts and Figures
2) Kothari *et al, Clin Transl Med* 2014, 3(35)
3) Tsai *et al, Genes Dev* 2013, 27: 2192-2206
4) Bartel, *Cell* 2004, 116: 281-297
5) Gregory *et al, Cell Cycle, 2009*
6) Kundu *et al, Oncogene* 2015, 1-14

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