Critical review

Human

Regulatory interplay between microRNAs and Snail family proteins in cancer

Y Chen[1]*, T Gridley[2]

Abstract

Introduction

The Snail gene family encodes evolutionarily conserved transcriptional repressor proteins that bind to E box sequences. In mammals, the SNAI1 and SNAI2 proteins are key regulators of the epithelial–mesenchymal transition both during embryonic development and tumour metastasis. Recent work has shown that SNAI1/SNAI2 gene and protein expression is regulated during tumourigenesis by a number of microRNAs. Furthermore, the existence of double-negative feedback loops that provide exquisite regulation of SNAI1/SNAI2 expression and the expression of microRNAs binding to the *SNAI1* and *SNAI2* mRNAs has been revealed.

Here, we review new studies highlighting the regulatory interplay of miRNA expression, regulation of SNAI1 and SNAI2 expression and the epithelial–mesenchymal transition in the progression and metastasis of ovarian and other epithelial tumours. We also summarise new data demonstrating a role for SNAI1 and SNAI2 expression in mediating acquired tumour drug resistance.

Conclusion

This review highlights the important roles played by the transcriptional repressors SNAI1 and SNAI2 during tumour progression and metastasis, as well as their regulatory interplay

* Corresponding author
Email: Ying.Chen@tufts.edu

[1] Department of Biomedical Engineering, Tufts University, Medford, MA 02155, USA
[2] Center for Molecular Medicine, Maine Medical Center Research Institute, Scarborough, ME 04074, USA

with the expression of multiple different microRNAs.

Introduction

Snail gene family members encode highly conserved zinc finger transcriptional repressor proteins[1,2]. In mammals, the Snail family genes include *SNAI1* (also known as Snail), *SNAI2* (Slug) and *SNAI3* (Smuc). All Snail family proteins contain four or five tandem zinc finger sequence-specific DNA-binding domains capable of recognising E2 box sequences (CAGGTG and CACCTG). Snail family proteins are critical for diverse processes during both embryonic development and disease. The SNAI1 and SNAI2 proteins are key regulators of the epithelial–mesenchymal transition (EMT) and also have demonstrated roles in other important developmental and cellular processes, such as the protection of cells from programmed cell death, the establishment of left–right asymmetry and the regulation of cell motility.

MicroRNAs (miRNAs) are a class of short (approximately 22 nucleotide) non-coding RNA molecules that play important regulatory roles in a wide variety of cellular processes, including cell proliferation, differentiation, apoptosis, senescence and tumourigenesis[3,4]. miRNAs negatively regulate gene expression post-transcriptionally by binding to the 3'-untranslated region (UTR) of their target mRNAs to inhibit their translation or decrease mRNA stability. It has been estimated computationally that more than 60% of protein-coding genes may be miRNA targets[3].

EMT is a cellular programme during which epithelial cells lose epithelial characteristics and acquire mesenchymal traits[5]. EMT occurs normally during embryonic development, but also occurs during pathological processes such as fibrosis and tumour metastasis[6,7]. During EMT, epithelial cells disassemble intercellular junctional complexes, lose apical-basal polarity and adherence to the basement membrane and gain a more fibroblast-like morphology with increased motility and invasiveness. Several transcription factors, including SNAI1, SNAI2, ZEB1 and ZEB2, are key regulators of the EMT. In addition, recent studies have established that various miRNAs also regulate EMT[7]. In this review, we summarise new studies highlighting the regulatory interplay of miRNA expression, regulation of SNAI1/SNAI2 expression and the EMT in the progression and metastasis of ovarian and other epithelial tumours. We also summarise new data demonstrating a role for SNAI1 and SNAI2 expression in mediating acquired tumour drug resistance.

Discussion

miRNA regulation of SNAI1/SNAI2 in ovarian cancer

Ovarian cancer is the fifth leading cause of cancer death in women in the United States and is the most lethal gynaecologic malignancy. The Cancer Genome Atlas (TCGA) Research Network has catalogued mRNA expression, miRNA expression, promoter DNA methylation and DNA copy number alterations in a large set of different tumour types, including 489 high-grade serous ovarian adenocarcinomas[8]. They used non-negative matrix factorisation consensus clustering to define four gene expression subtypes of high-grade serous

Critical review

ovarian cancer, termed immunoreactive, differentiated, proliferative and mesenchymal. Survival duration did not differ significantly among patients with tumours of these different gene expression subtypes. Similar cluster analysis of miRNA expression defined three expression subtypes. The miRNA subtype 1 partially overlapped the mRNA proliferative subtype and miRNA subtype 2 partially overlapped the mRNA mesenchymal subtype. Survival duration differed significantly between these miRNA subtypes. Patients with miRNA subtype 1 tumours survived significantly longer than patients with tumours of the other two subtypes. While patient survival did not correlate with any of the four gene expression subtypes, a 193-gene transcriptional signature predictive of overall survival could be defined using an integrated expression data set from 215 samples. Subsequent analyses of the TCGA data of miRNA expression of serous ovarian tumours demonstrated that expression of a set of 34 miRNAs also was predictive of overall patient survival[9].

The TCGA Research Network has recently described prognostically relevant gene signatures for high-grade serous ovarian cancer[10]. Together, these subtype and survival gene expression signatures provide a prognostic model of high-grade serous ovarian cancer classification, which they term 'Classification of Ovarian Cancer' (CLOVAR). In this study, the descriptions of the previously observed mesenchymal, differentiated, proliferative and immunoreactive subtypes of high-grade serous ovarian cancer were substantially expanded. During analysis, these subtype signatures were combined with the CLOVAR survival signature, containing genes whose expression was either strongly correlated (good prognosis genes) or anti-correlated (poor prognosis genes) with survival. The detailed stratification permitted by the new classifications resulted in the identification of

a subset of high-grade serous ovarian cancer, with only 23 months median survival and 63% platinum therapy resistance, compared with median survival of 46 months and a platinum resistance rate of 23% in all other cases. These worst outcome tumours were classified as both CLOVAR poor prognosis (for survival) and CLOVAR mesenchymal subtype, which was enriched for EMT-related genes[10].

Another group recently described a set of integrated genomic analyses that further defined the mesenchymal gene expression subtype of serous ovarian cancer defined by the TCGA analysis and its relationship to the EMT. Integration of the TCGA mRNA, miRNA, DNA copy number and DNA methylation data revealed a miRNA-regulatory network that defined an integrated mesenchymal (iM) subtype associated with poor overall survival in 459 cases of serous ovarian cancer from the TCGA cohort and 560 cases from independent cohorts[11]. In these analyses, 219 genes were predicted to be targets of 19 miRNAs, including genes encoding the EMT inducers SNAI2 and ZEB2. Clustering analyses based on the 219 miRNA-associated genes identified two clusters in both the TCGA cohort and in three independent patient cohorts. These clusters were termed the iM subtype and the integrated epithelial (iE) subtype. Patients with tumours of the iM subtype had significantly shorter overall survival than patients with tumours of the iE subtype[11]. Histological features of the tumours also differed between the iM and iE subtypes. Eight key miRNAs, including miR-506, miR-141 and miR-200a, were predicted to regulate 89% of the targets in the iM network (Figure 1). The least studied of these miRNAs was miR-506. Functional assays in ovarian cancer cell lines demonstrated that miR-506 bound the 3' UTR of the *SNAI2* mRNA, causing increased E-cadherin expression, inhibited cell migration and invasion and inhibition of TGFβ-induced EMT.

Nanoparticle delivery of miR-506 in orthotopic mouse xenograft models resulted in E-cadherin induction and reduced tumour growth, suggesting that miR-506 delivery may be a useful therapeutic strategy for serous ovarian cancer[11].

Another recent study supports the hypothesis that induction of the EMT programme is important for ovarian cancer progression and metastasis. Transcriptome and pathway analyses were performed on 14 matched sets of primary and metastatic serous ovarian cancer samples collected from seven patients with Stage III/IV cancer[12]. Unsupervised hierarchical clustering revealed that the metastatic samples of five of the seven patients grouped closely with their respective primary samples, while the metastatic samples of the other two patients clustered most closely with one another, distant from their respective primary samples. Pathway analysis showed that 13 of the 20 most significantly enriched pathways were associated with the EMT. Further analyses focusing on EMT-associated genes clearly distinguished the primary from the metastatic samples in six of the seven patients[12].

A siRNA screen identified the homeobox transcription factor ALX1 as a novel regulator of EMT in ovarian cancer[13]. siRNA-mediated attenuation of *ALX1* expression restored E-cadherin expression and cell–cell junction formation in ovarian cancer cells, resulting in suppression of cell invasion, anchorage-independent growth and tumour formation. Conversely, overexpression of ALX1 in ovarian cancer cells or non-tumourigenic epithelial cells induced EMT. ALX1 expression upregulated *SNAI1* expression, and siRNA-mediated *SNAI1* knockdown in ALX1-overexpressing cells demonstrated that SNAI1 upregulation mediated the EMT activation and cell invasion exhibited by ALX1-expressing cells[13].
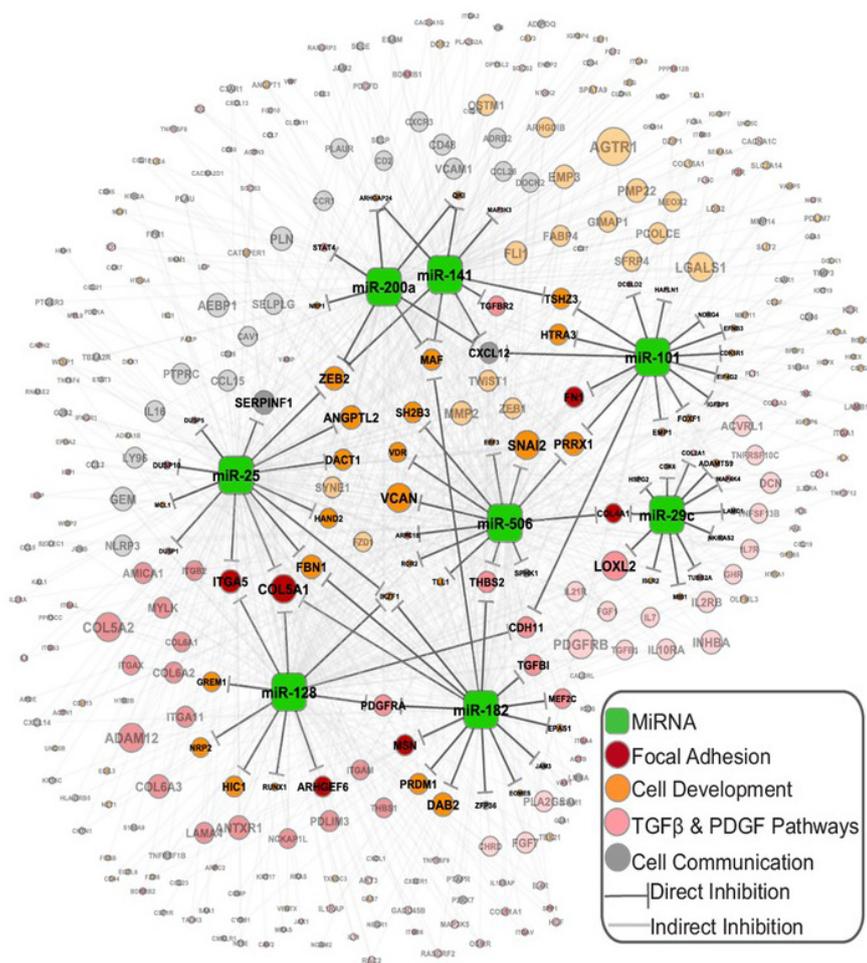
Figure 1: miRNA–gene interaction network of eight key miRNAs and the EMT-related genes they are predicted to regulate in serous ovarian cancer cells. Reproduced with permission from Yang et al.[11].

Another recent report determined the effects of *SNAI1* and *SNAI2* over-expression and knockdown in the SKOV3 ovarian carcinoma cell line[14]. *SNAI1* overexpression increased cell proliferation, migration, invasion and clonogenicity in cell culture assays, while shRNA-mediated *SNAI1* knockdown reduced these parameters. In a mouse xenograft assay, *SNAI1*-overexpressing SKOV3 cells exhibited higher rates of tumour growth and larger tumour volumes than control SKOV3 cells. Interestingly, none of these effects were observed in SKOV3 cells with SNAI2 knockdown or overexpression.

SNAI1/SNAI2 and miRNA regulation in other epithelial cancers

Similar modes of regulation of SNAI1/SNAI2 expression are utilised in other cancer types. Breast cancer is the most commonly diagnosed cancer in women and is the second leading cause of death in women. MiR-124 expression was significantly suppressed in human breast cancer specimens, and expression levels were inversely correlated with the histological grade of the cancer[15]. Ectopic expression of miR-124 in the MDA-MB-231 and BT-549 breast cancer cell lines strongly inhibited

cell motility and invasive capacity, as well as the EMT programme. Luciferase reporter assays demonstrated that the *SNAI2* mRNA was a direct target of mir-124 in these cells. *SNAI2* knockdown impaired the motility of MDA-MB-231 cells, whereas *SNAI2* re-expression abrogated the reduction in motility and invasion ability induced by miR-124 in MDA-MB-231 cells. In a prostate cancer xenograft model, a recent study has demonstrated that transcriptional induction of *SNAI2* expression was essential for cyclin D1b–mediated proliferative and invasive properties, implicating SNAI2 as a critical driver of disease progression. Expression of cyclin D1b, a splice variant of the cell cycle regulator cyclin D1, was highly correlated with SNAI2 expression in clinical samples of advanced disease. *In vivo* analyses provided strong evidence that SNAI2 enhanced both tumor growth and metastatic phenotypes[16].

miRNA regulation of SNAI2 expression may be involved in the early progression to malignancy and metastasis during prostate cancer. miR-182 and miR-203 coordinately regulate SNAI2 expression in a premalignant prostate cell EMT model consisting of the prostate primary epithelial cell line EP156T and progeny mesenchymal EPT1 cells[17]. miR-182 and miR-203 expression was completely repressed during EMT from EP156T cells to the mesenchymal EPT1 cells. The 3'-UTR of the *SNAI2* mRNA contains two evolutionarily conserved binding sites specific for miR-182 and miR-203. Striking overlap of the affected genes between EPT1 cells re-expressing miR-182/203 or with shRNA-mediated knockdown of SNAI2 suggested that readout of miR-182 and miR-203 function in EPT1 cells was primarily manifested by repressing SNAI2 protein expression. This study further identified the P-cadherin (*CDH3*) gene as a direct target for transcriptional repression by the SNAI2 protein[17].
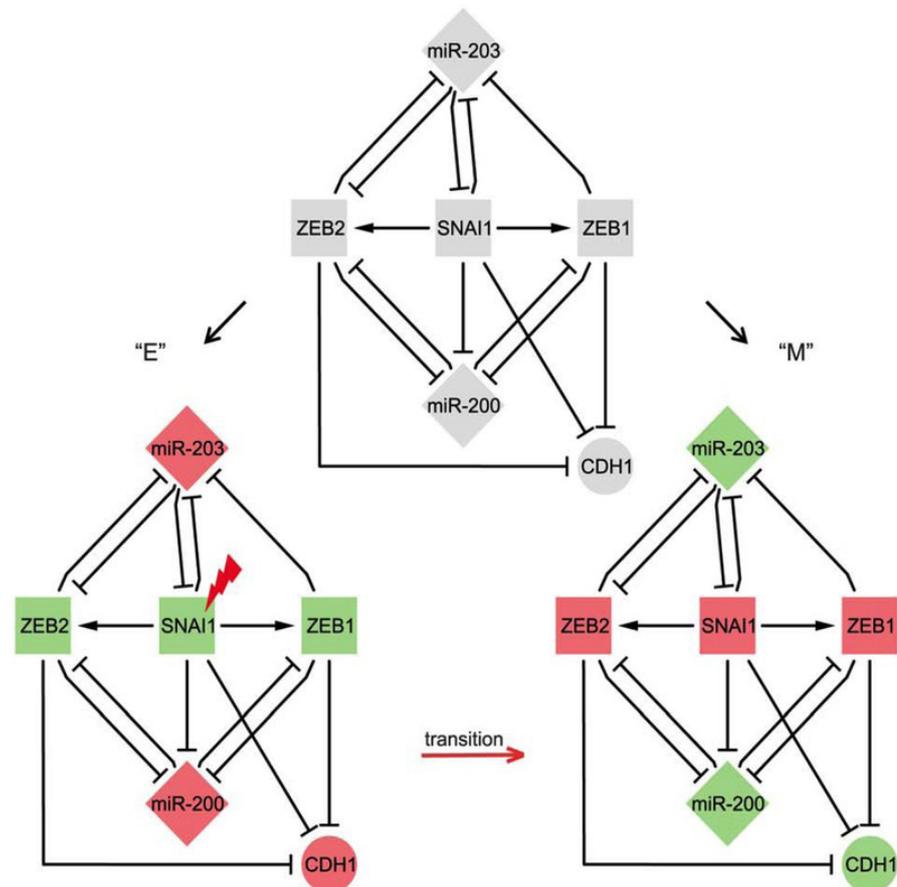
SNAI1/SNAI2-miRNA double-negative feedback loops

A recently revealed aspect of the regulation of *SNAI1/SNAI2* gene expression during tumourigenesis and metastasis is the existence of double-negative feedback loops regulating expression of the *SNAI1* and *SNAI2* genes and the miRNAs binding to the *SNAI1* and *SNAI2* mRNAs. A double-negative feedback loop involving the miR-200 family and the ZEB1 and ZEB2 genes in the control of EMT has been described previously[18,19]. Double-negative feedback loops can behave as a bistable switch in which a system can reversibly transition between two alternative steady states (for example, as epithelial or mesenchymal cells)[20,21].

In a mouse model of progressive prostate cancer, the *SNAI2* gene is involved in a double-negative feedback loop with both miR-1 and the miR-200 family[22]. The SNAI2 protein is a direct repressor of miR-1 and miR-200 transcription, binding to E2 box sites in the promoters of these miRNAs. The miR-1 and miR-200 miRNAs, in turn, directly bind the 3'-UTR of the *SNAI2* mRNA to negatively regulate its stability or translation. Similar double-negative feedback loops have been described between the miR-34 family and the *SNAI1* gene in human colon cancer cell lines[23], and between miR-203 and both the *SNAI1*[24] and *SNAI2*[25] genes. Computational modelling of a core network integrating expression of the miR-203/SNAI1 negative feedback loop with the miR-200/ZEB1/ZEB2 loop revealed the existence of stable mesenchymal and epithelial states for this network (Figure 2) and suggested that the miR-203/SNAI1 feedback loop plays a crucial role in transitions between states and in the stabilisation of the core network in the mesenchymal and epithelial states[24].

SNAI1 and SNAI2 expression may mediate tumour drug resistance

SNAI1 and SNAI2 expression may also play a role in a tumour's acquisition of resistance to chemotherapy.

Figure 2: Integration of the miR-203/SNAI1 and miR-200/ZEB1/ZEB2 double-negative feedback loops. The top panel shows the core network integrating described interactions between miR-203, the miR-200 family, SNAI1, ZEB1, ZEB2 and E-cadherin (CDH1). The bottom panels show the stable epithelial (E) and mesenchymal (M) states obtained after dynamic analyses. Green indicates downregulated expression; red indicates upregulated expression. The red "lightning bolt" in the lower left panel indicates SNAI1 upregulation triggering the transition from the epithelial to the mesenchymal state. Reproduced with permission from Moes et al.[24].

Standard treatment for ovarian cancer involves tumour debulking surgery followed by platinum-taxane chemotherapy. Within 6 months, platinum-resistant tumours recur in approximately 25% of patients. Analysis of the A2780 ovarian adenocarcinoma cell line and a cisplatin-resistant variant revealed that the cisplatin-resistant cells exhibited a more mesenchymal morphology, with spindle-shaped cells that extended pseudopodia[26]. The cisplatin-resistant cells exhibited increased motility and invasive behaviour, and siRNA-mediated knockdown of SNAI1 and/or SNAI2 expression in the cisplatin-resistant cells restored the epithelial morphology exhibited by the parental A2780 line, reduced cellular motility and invasion and increased sensitivity of the knockdown cells to cisplatin. Importantly, increased expression of the *SNAI1* and *SNAI2* genes, as well as several other genes involved in regulating the EMT (such as *TWIST1*, *TWIST2*, *ZEB1* and *ZEB2*),

OA Biology
Open Access

was observed in chemoresistant human primary ovarian tumours.

SNAI1 and SNAI2 expression had previously been shown to mediate resistance to radiation and paclitaxel in the A4 ovarian cancer cell line[27]. In these cells, this resistance was caused, at least in part, by SNAI1/SNAI2-mediated repression of the pro-apoptotic genes *PUMA*, *ATM* and *PTEN* during p53-mediated pro-survival signalling. Another recent study demonstrated that the anaesthetic propofol increased paclitaxel-induced apoptosis of several ovarian cancer cell lines[28]. Paclitaxel sensitivity of the cell lines correlated with basal SNAI2 expression levels, and propofol treatment reduced SNAI2 expression. These studies indicate that upregulation of SNAI1 and SNAI2 expression in ovarian cancer cells may contribute to multiple aspects of tumour resistance to therapeutic treatment and suggest that treatments to reduce SNAI1/SNAI2 expression may be a useful therapeutic strategy.

Conclusion

This review highlights the important roles played by the transcriptional repressors SNAI1 and SNAI2 during tumour progression and metastasis, as well as their regulatory interplay with expression of multiple different miRNAs. The identification of double-negative feedback loops connecting transcription of various miRNAs and the EMT transcriptional regulators *SNAI1*, *SNAI2*, *ZEB1* and *ZEB2* have revealed a powerful regulatory system for potentially reversible transitions between the epithelial and mesenchymal states and highlight potential targets for therapeutic intervention in tumour progression, metastasis and acquisition of drug resistance.

Abbreviations list

CLOVAR, Classification of Ovarian Cancer; EMT, epithelial–mesenchymal transition; iE, integrated epithelial; iM, integrated mesenchymal; miRNA, microRNA; TCGA, The Cancer Genome Atlas; UTR, untranslated region.

Acknowledgement

References

1. Barrallo-Gimeno A, Nieto MA. Evolutionary history of the Snail/Scratch superfamily. Trends Genet. 2009 Jun;25(6): 248–52.
2. Chiang C, Ayyanathan K. Snail/Gfi-1 (SNAG) family zinc finger proteins in transcription regulation, chromatin dynamics, cell signaling, development, and disease. Cytokine Growth Factor Rev. 2013 Apr;24(2):123–31.
3. Ebert MS, Sharp PA. Roles for microRNAs in conferring robustness to biological processes. Cell. 2012 Apr;149(3):515–24.
4. Mendell JT, Olson EN. MicroRNAs in stress signaling and human disease. Cell. 2012 Mar;148(6):1172–87.
5. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell. 2009 Nov;139(5):871–90.
6. Scheel C, Weinberg RA. Cancer stem cells and epithelial-mesenchymal transition: concepts and molecular links. Semin Cancer Biol. 2012 Oct;22(5):396–403.
7. De Craene B, Berx G. Regulatory networks defining EMT during cancer initiation and progression. Nat Rev Cancer. 2013 Feb;13(2):97–110.
8. Network TCGAR. Integrated genomic analyses of ovarian carcinoma. Nature. 2011 Jun;474(7353):609–15.
9. Creighton CJ, Hernandez-Herrera A, Jacobsen A, Levine DA, Mankoo P, Schultz N, et al. Integrated analyses of microRNAs demonstrate their widespread influence on gene expression in high-grade serous ovarian carcinoma. PLoS One. 2012;7(3):e34546.
10. Verhaak RG, Tamayo P, Yang JY, Hubbard D, Zhang H, Creighton CJ, et al. Prognostically relevant gene signatures of high-grade serous ovarian carcinoma. J Clin Invest. 2013 Jan;123(1):517–25.
11. Yang D, Sun Y, Hu L, Zheng H, Ji P, Pecot CV, et al. Integrated analyses identify a master microRNA regulatory network for the mesenchymal subtype in serous ovarian cancer. Cancer Cell. 2013 Feb;23(2):186–99.
12. Lili LN, Matyunina LV, Walker LD, Wells SL, Benigno BB, McDonald JF. Molecular profiling supports the role of epithelial-to-mesenchymal transition (EMT) in ovarian cancer metastasis. J Ovarian Res. 2013 Jul;6(1):49.
13. Yuan H, Kajiyama H, Ito S, Yoshikawa N, Hyodo T, Asano E, et al. ALX1 induces snail expression to promote epithelial-to-mesenchymal transition and invasion of ovarian cancer cells. Cancer Res. 2013 Mar;73(5):1581–90.
14. Lu ZY, Dong R, Li D, Li WB, Xu FQ, Geng Y, et al. SNAI1 overexpression induces stemness and promotes ovarian cancer cell invasion and metastasis. Oncol Rep. 2012 May;27(5):1587–91.
15. Liang YJ, Wang QY, Zhou CX, Yin QQ, He M, Yu XT, et al. MiR-124 targets Slug to regulate epithelial-mesenchymal transition and metastasis of breast cancer. Carcinogenesis. 2013 Mar;34(3): 713–22.
16. Augello MA, Burd CJ, Birbe R, McNair C, Ertel A, Magee MS, et al. Convergence of oncogenic and hormone receptor pathways promotes metastatic phenotypes. J Clin Invest. 2013 Jan;123(1): 493–508.
17. Qu Y, Li WC, Hellem MR, Rostad K, Popa M, McCormack E, et al. MiR-182 and miR-203 induce mesenchymal to epithelial transition and self-sufficiency of growth signals via repressing SNAI2 in prostate cells. Int J Cancer. 2013 Aug;133(3):544–55.
18. Brabletz S, Brabletz T. The ZEB/miR-200 feedback loop—a motor of cellular plasticity in development and cancer? EMBO Rep. 2010 Sep;11(9):670–7.
19. Hill L, Browne G, Tulchinsky E. ZEB/miR-200 feedback loop: at the crossroads of signal transduction in cancer. Int J Cancer. 2013 Feb;132(4):745–54.
20. Ferrell JE, Jr. Self-perpetuating states in signal transduction: positive feedback, double-negative feedback and bistability. Curr Opin Cell Biol. 2002 Apr;14(2): 140–8.
21. Cai S, Zhou P, Liu Z. Functional characteristics of a double negative feedback loop mediated by microRNAs. Cogn Neurodyn. 2013 Oct;7(5):417–29.
22. Liu YN, Yin JJ, Abou-Kheir W, Hynes PG, Casey OM, Fang L, et al. MiR-1 and miR-200 inhibit EMT via Slug-dependent and tumorigenesis via Slug-independent

OA Biology
Open Access

Critical review

mechanisms. Oncogene. 2013 Jan;32(3): 296–306.

23. Siemens H, Jackstadt R, Hunten S, Kaller M, Menssen A, Gotz U, et al. miR-34 and SNAIL form a double-negative feedback loop to regulate epithelial-mesenchymal transitions. Cell Cycle. 2011 Dec;10(24):4256–71.

24. Moes M, Le Bechec A, Crespo I, Laurini C, Halavatyi A, Vetter G, et al. A novel network integrating a miRNA-203/SNAI1 feedback loop which regulates epithelial to mesenchymal transition. PLoS One. 2012;7(4):e35440.

25. Ding X, Park SI, McCauley LK, Wang CY. Signaling between transforming growth factor beta (TGF-beta) and transcription factor SNAI2 represses expression of microRNA miR-203 to promote epithelial-mesenchymal transition and tumor metastasis. J Biol Chem. 2013 Apr;288(15):10241–53.

26. Haslehurst AM, Koti M, Dharsee M, Nuin P, Evans K, Geraci J, et al. EMT transcription factors snail and slug directly contribute to cisplatin resistance in ovarian cancer. BMC Cancer. 2012 Mar; 12:91.

27. Kurrey NK, Jalgaonkar SP, Joglekar AV, Ghanate AD, Chaskar PD, Doiphode RY, et al. Snail and slug mediate radioresistance and chemoresistance by antagonising p53-mediated apoptosis and acquiring a stem-like phenotype in ovarian cancer cells. Stem Cells. 2009 Sep;27(9):2059–68.

28. Wang P, Chen J, Mu LH, Du QH, Niu XH, Zhang MY. Propofol inhibits invasion and enhances paclitaxel- induced apoptosis in ovarian cancer cells through the suppression of the transcription factor slug. Eur Rev Med Pharmacol Sci. 2013 Jul;17(13):1722–9.

For citation purposes: *Chen Y, Gridley T.* Regulatory interplay between microRNAs and Snail family proteins in cancer. OA Biology 2014 Jan 18;2(1):1.