RNA & DISEASE 2014; 1: e345. doi: 10.14800/rd.345; © 2014 by Wassim Abou-Kheir, *et al*.
http://www.smartscitech.com/index.php/rd

REVIEW

Bone metastatic prostate cancer and resistance to tyrosine kinase inhibitors: an intimate relationship between loss of miR-203 and up-regulation of EGFR signaling

Wassim Abou-Kheir[1], Tarek H. Mouhieddine[1], Muhieddine M. Itani[1], Yen-Nien Liu[2]

[1]*Department of Anatomy, Cell Biology and Physiological Sciences, Faculty of Medicine, American University of Beirut, Beirut 1107-2020, Lebanon*
[2]*Graduate Institute of Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, Taipei 11503, Taiwan*

Correspondence: Wassim Abou-Kheir or Yen-Nien Liu
E-mail: wa12@aub.edu.lb or liuy@tmu.edu.tw
Received: May 15, 2014
Published: November 17, 2014

A tumor suppressor role for miR-203 in RAS-dependent prostate cancer metastasis has been described recently by our group. We have explored the regulatory mechanisms by which miR-203 is being regulated through EGFR signaling. We investigated the molecular mechanism of metastasis and identified novel roles of genes that interact with miR-203 downstream of activated Ras. We showed an amplifying regulatory loop involving the direct interaction of miR-203 with the EGFR ligands, AREG, EREG, and TGFA 3'UTR. Using clinical specimens and database analysis, our data suggested that decreasing miR-203 and increasing EGFR ligands, AREG, EREG, and TGFA expressions are correlated with prostate cancer progression. Since tyrosine kinase inhibitors (TKIs) have been shown to inhibit tyrosine phosphorylation of EGFR in a dose-dependent manner, we examined a role for miR-203 in TKIs-induced apoptosis in RAS-activated prostate cancer. We investigated the mechanisms by which miR-203 overexpression contributes to TKIs-resistant RAS-activated prostate cancer cells apoptosis. We have shown indications for candidate miR-203 targets that are either influenced by anti-apoptotic proteins (e.g. API5, BIRC2, and TRIAP1) or positively influenced by a novel NF-κB-inducible oncogenic molecule, TNFAIP8. These observations suggest that the latter category may be synergistically affected by the regulatory loop of miR-203 depletion and anti-apoptotic proteins overexpression. Our results provided evidence showing a role of miR-203 in regulating the expression of EGFR signaling genes in response to TKIs-resistance during prostate cancer progression.

Keywords: metastatic prostate cancer; Ras, miR-203; EGFR; TKI-resistance

To cite this article: Wassim Abou-Kheir, *et al*. Bone metastatic prostate cancer and resistance to tyrosine kinase inhibitors: an intimate relationship between loss of miR-203 and up-regulation of EGFR signaling. RNA Dis 2014; 1: e345. doi: 10.14800/rd.345.

Prostate cancer (PC) is the most commonly diagnosed cancer and the second most common cause of death among men in the United States [1]. PC is usually androgen-dependent and thus, androgen deprivation therapy has been the standard treatment of PC for a long time. However, after a few years of treatment, PC may become resistant to such treatment, rendering it highly incurable due to the high recurrence rate. Furthermore, the fatality of PC lies in its drug resistance and vigorous ability to metastasize to distant organs such as bones, lymph nodes, liver, lungs and the brain [2]. It is imperative to understand the various mechanisms responsible for the progression of PC in order to

devise more effective targeted therapies.

Prostate cancer has been mostly linked to the epidermal growth factor receptor (EGFR) dysfunction, which upon activation induces cell survival, proliferation, invasion, metastasis, and thus, drug resistance [3, 4]. Furthermore, PC drug resistance could stem out from downstream EGFR genetic mutations, such as the KRAS, which in turn could activate the EGFR in an autocrine manner [5, 6].

Current research is focusing a lot on the domain of microRNAs, which has been found to influence many physiological and pathological conditions. The exact mechanism by which microRNAs are able to initiate a pathological state is still unclear, but specific microRNAs have been studied and their presence or absence has been associated with certain types of cancers. Our microRNA of interest, miR-203, has been proposed to have tumor-suppressive functions in different types of cancers, including lung, breast and skin cancer [7-9]. Moreover, a down-regulation of miR-203 has been specifically implicated in bone metastatic PC, whether it be in cell lines or clinical specimens [10]. Yet, the way by which miR-203 modulates EGFR signaling is still unclear.

Therefore, we aimed to study miR- 203 expression in PC cell lines and human specimens, and its effect in suppressing bone metastasis and inducing apoptosis in tyrosine kinase inhibitors (TKI)-resistant Ras-activated PC cells in which miR-203 is down-regulated by EGFR signaling[11]. We found that miR-203 directly targets the 3'UTR of the mRNAs of EGFR ligands (*EREG* and *TGFA*) and anti-apoptotic proteins (*API5, BIRC2*, and *TRIAP1*). In addition, inhibiting miR-203 induced both cell invasion and TKI resistance in PC cells, meaning that miR-203 has a prominent role in the EGFR network. We conclude that PC tumors with down-regulated expression of miR-203 have an increase in EGFR ligands (*EREG* and *TGFA*) and anti-apoptotic proteins (*API5, BIRC2,* and *TRIAP1*) expression, leading to PC bone metastasis and TKIs resistance.

RAS-dependent signaling has been found to contribute to the aggressiveness, metastasis, tumor grade and prognosis of PC [12, 13], where DU145, a human non-metastatic prostate cancer cell line, has been shown to rapidly form larger metastatic lesions when having a RasG37 mutation [14]. To make sure if there was any association between miR-203 and Ras signaling, we studied the expression of miR-203 and its associated mRNAs in a publicly available data set of 99 primary tumors and 13 distant metastasis tissue specimens collected and analyzed at Memorial Sloan-Kettering Cancer Center [15]. According to an analysis of summed z-scores, we found that miR-203 was down-regulated in metastatic specimens and samples with altered KRAS signaling, while in samples with high expression of miR-203, EGFR pathway gene signatures were down-regulated. Furthermore, we examined the expression of miR-203 in DU145 cells harboring different Ras mutations and found that it was mostly reduced in the bone-derived RasB1 clone. Moreover, we found an increased p-EGFR and p-ERK1/2 expression in cells with a Ras mutation, which was reversed when we forced the overexpression of miR-203 precursor in RasB1 and LNCap cells.

We further assessed the effect of overexpressing miR-203 in Ras-mutated PC cells lines. We found a reduced invasion and bone metastasis potential in RasB1 and reduced cell growth and invasion in DU145 cell lines. Furthermore, we found that the mRNA levels of the EGFR ligands, AREG, EREG, and TGFA, were up-regulated in RasB1, indicating increased autocrine activation. Upon treatment with EGF and EGFR inhibitor (CI1033), the EGFR ligands were increased and decreased, respectively. On the other hand, miR-203 was increased upon treatment with CI1033 and decreased upon EGF treatment. We proposed the presence of a miR-203 binding site on the above-mentioned EGFR ligands, since their expression is decreased in the presence of miR-203 and decreased luciferase activities were detected upon co-transfection with miR-203 precursor by reporter assay in RasB1 cells. Moreover, this effect was ameliorated and 3'UTR activity of the ligands was induced in DU145 following miR-203 inhibition.

Next, we analyzed 25 independent prostate tumors collected from Wang Fang Hospital, Taipei Medical University, Taiwan, to check if our in vitro findings coincide with human specimens. Upon dividing the specimens into two groups of high or low expression of EREG and TGFA, we found that there was an inverse correlation between miR-203 and EREG and TGFA expression, but not AREG. Incidentally, specimens with high AREG, EREG and TGFA expression were metastatic tumors and had higher KRAS oncogenic response gene signatures.

We then sought to uncover the role of miR-203 in mediating apoptosis in TKI-resistant Ras-activated PC cells. We found that RasB1 cells lose their resistance to death via EGFR inhibitors when transfected with miR-203 and DU145 cells gain resistance via activating the EGFR pathway when transfected with anti-miR-203. Furthermore, miR-203 over-expression was associated with a TKI-induced increase in caspase-3/7 in RasB1 cells, which was not the case in DU145 cells. The next step was an in vivo experiment where overexpressing miR-203 in RasB1 cells led to an increased sensitivity to TKI-induced apoptosis and a decrease in tumor growth in nude mice, following 3 weeks of CI1033

treatment. This means that miR-203 may control various apoptotic or anti-apoptotic genes related to the EGFR signaling pathway.

To validate the ability of miR-203 to modulate anti-apoptotic pathways, we studied the effect of EGF on RasB1 cells and found that it increased the mRNA expression of the anti-apoptotic genes API5, BIRC2and TRIAP1, whereas miR-203 precursor decreased them. Furthermore, the presence of anti-miR-203 in DU145 decreased the mRNA expression of API5, BIRC2 and TRIAP1. In addition to that, miR-203 was found to regulate the NF-kB-inducible, oncogenic TNFAIP8 molecule.

All in all, our study suggests that miR-203 is an important mediator in the progression and aggressiveness of prostate cancer. It has been shown to modulate downstream genes of the EGFR signaling pathway (EREG and TGFA), apoptotic genes (API5, BIRC2 and TIAP1) and other genes such as TNFAIP8. This adds to the multitude of studies that are delving deeper into the world of microRNAs and discovering that these tiny genetic pieces are capable of directing many physiological and pathological states. This is why we continued our search for more microRNAs involved in prostate cancer production and augmentation.

Our focus remained on alterations in the Ras signaling pathway. According to the literature, it has been shown that activation of the Ras pathway in PC is correlated with alterations in other pathways such as the mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)/AKT cascades [16, 17], and WNT signaling [18]. Interestingly, aberrant WNT signaling may develop through accumulated β-catenin that associates with members of the T-cell factor (TCF) and lymphoid enhancer factor (LEF) in the nucleus [19], which in turn activate the c-Myc [20], c-jun, metalloproteinase matrilysin [21] and cyclin D1 genes [22]. It is hypothesized that aggressive PC is the result of activated WNT signaling via the loss of regulation of transcription factor 7 (TCF7) expression and β-catenin accumulation. Of interest, microRNAs have been found to regulate the WNT-β-catenin pathway by binding to the 3'UTR region [23]. Specifically, miR-34a has been implicated in malignant PC and has shown anti-proliferative and apoptotic effects [24, 25]. Thus, we are currently seeking to understand the tumor suppressive role of miR-34a in PC in modulating the canonical WNT pathway.

In short, out results reveal that reduced miR-34a expression is accompanied with activated WNT signaling genes, specifically in PC that metastasizes to the bone (cell lines and human specimens). On the other hand, increasing miR-34a expression reduces TCF7 expression, bone metastasis and proliferation and increases the anti-apoptotic BIRC5 gene expression in cancer cell lines. For this reason, we postulate that miR-34a deficiency is required for bone metastasis and the anti-apoptotic ability of Ras-mutated PC cells in which the WNT/TCF7 pathway and BIRC5 are activated.

With more research, we are beginning to understand the intricate mechanisms underlying the production and progression of cancerous cells, at the level of microRNAs. We have shown that bone metastasis and TKI resistance in prostate cancer could be mediated via the loss of miR-203, which in turn leads to EGFR signaling dysregulation. A further study also led us to believe that the aggressiveness of prostate cancer could also be mediated through another microRNA, the miR-34a, where its loss would lead to the activation of the WNT/TCF7 signaling pathway. These microRNAs, and others to be discovered, are promising therapeutic targets and could be the road to finally overcoming treatment resistant aggressive prostate cancer. More research is needed for the whole interaction map to come into picture and be able to target individual microRNAs in an individualized course of treatment.

Acknowledgments

This work was jointly supported by grants from the National Health Research Institutes (NHRI-EX103-10308BC) and the Ministry of Science and Technology (MOST 103-2314-B-038 -051) of Taiwan to Y-N Liuand by funding from the Medical Practice Plan-MPP (AUB-FM) and the Lebanese National Council for Scientific Research (CNRS) to W. Abou-Kheir.

References

1. Denmeade SR, Isaacs JT.Development of prostate cancer treatment: the good news.The Prostate 2004;58:211-224.
2. Gleave M, Hsieh JT, Gao CA, von Eschenbach AC, Chung LW.Acceleration of human prostate cancer growth in vivo by factors produced by prostate and bone fibroblasts.Cancer research 1991;51:3753-3761.
3. Di Lorenzo G, Tortora G, D'Armiento FP, De Rosa G, Staibano S, Autorino R *et al*. Expression of epidermal growth factor receptor correlates with disease relapse and progression to androgen-independence in human prostate cancer.Clinical cancer research : an official journal of the American Association for Cancer Research 2002;8:3438-3444.
4. Traish AM, Morgentaler A.Epidermal growth factor receptor expression escapes androgen regulation in prostate cancer: a potential molecular switch for tumour growth.British journal of cancer 2009;101(12):1949-1956.
5. Nazarian R, Shi H, Wang Q, Kong X, Koya RC, Lee H *et al*.Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation.Nature 2010;468:973-977.

6. Schulze A, Nicke B, Warne PH, Tomlinson S, Downward J.The transcriptional response to Raf activation is almost completely dependent on Mitogen-activated Protein Kinase Kinase activity and shows a major autocrine component.Molecular biology of the cell 2004;15:3450-3463.

7. Garofalo M, Romano G, Di Leva G, Nuovo G, Jeon YJ, Ngankeu A *et al*. EGFR and MET receptor tyrosine kinase-altered microRNA expression induces tumorigenesis and gefitinib resistance in lung cancers.Nature medicine 2012;18:74-82.

8. 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**:e35440.

9. Sonkoly E, Loven J, Xu N, Meisgen F, Wei T, Brodin P *et al*.MicroRNA-203 functions as a tumor suppressor in basal cell carcinoma.Oncogenesis 2012;1:e3.

10. Saini S, Majid S, Yamamura S, Tabatabai L, Suh SO, Shahryari V*et al*. Regulatory Role of mir-203 in Prostate Cancer Progression and Metastasis.Clinical cancer research : an official journal of the American Association for Cancer Research 2011;17:5287-5298.

11. Siu MK, Abou-Kheir W, Yin JJ, Chang YS, Barrett B, Suau F *et al*.Loss of EGFR signaling regulated miR-203 promotes prostate cancer bone metastasis and tyrosine kinase inhibitors resistance.Oncotarget 2014;5:3770-3784.

12. Mulholland DJ, Kobayashi N, Ruscetti M, Zhi A, Tran LM, Huang J *et al*. Pten loss and RAS/MAPK activation cooperate to promote EMT and metastasis initiated from prostate cancer stem/progenitor cells.Cancer research 2012;72:1878-1889.

13. Min J, Zaslavsky A, Fedele G, McLaughlin SK, Reczek EE, De Raedt T *et a*l. An oncogene-tumor suppressor cascade drives metastatic prostate cancer by coordinately activating Ras and nuclear factor-kappaB. Nature medicine 2010;16:286-294.

14. Yin JJ, Zhang L, Munasinghe J, Linnoila RI, Kelly K.Cediranib/AZD2171 inhibits bone and brain metastasis in a preclinical model of advanced prostate cancer. Cancer research 2010;70:8662-8673.

15. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS *et al*.Integrative genomic profiling of human prostate cancer. Cancer cell 2010;18:11-22.

16. Jiang H, Grenley MO, Bravo MJ, Blumhagen RZ, Edgar BA.EGFR/Ras/MAPK signaling mediates adult midgut epithelial homeostasis and regeneration in Drosophila. Cell Stem Cell 2011;8:84-95.

17. Roberts PJ, Der CJ.Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene 2007;26:3291-3310.

18. Anastas JN, Moon RT.WNT signalling pathways as therapeutic targets in cancer. Nat Rev Cancer 2013;13:11-26.

19. Polakis P.Wnt signaling and cancer. Genes Dev 2000;14:1837-1851.

20. He TC, Sparks AB, Rago C, Hermeking H, Zawel L, da Costa LT *et al*. Identification of c-MYC as a target of the APC pathway.Science 1998;281:1509-1512.

21. Crawford HC, Fingleton BM, Rudolph-Owen LA, Goss KJ, Rubinfeld B, Polakis P *et al*.The metalloproteinase matrilysin is a target of beta-catenin transactivation in intestinal tumors. Oncogene 1999;18:2883-2891.

22. Tetsu O, McCormick F.Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells.Nature 1999;398:422-426.

23. Catto JW, Alcaraz A, Bjartell AS, De Vere White R, Evans CP, Fussel S *et al*.MicroRNA in prostate, bladder, and kidney cancer: a systematic review. Eur Urol 2011;59:671-681.

24. Hermeking H.The miR-34 family in cancer and apoptosis. Cell Death Differ 2010;17:193-199.

25. Cheng CY, Hwang CI, Corney DC, Flesken-Nikitin A, Jiang L, Oner GM *et al*. miR-34 cooperates with p53 in suppression of prostate cancer by joint regulation of stem cell compartment. Cell Rep 2014;6:1000-1007.