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CORRESPONDENCE

Cellular and Molecular Biology

Reply to Comment on "miR-199b-5p-DDR1-ERK signalling axis suppresses prostate cancer metastasis via inhibiting epithelial-mesenchymal transition"

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We appreciate Ion Cristóbal and colleagues for their comments [1] on our paper recently published in *Br J Cancer* [2].

In our study [2], we assessed the prognostic significances of miR-199b-5p and DDR1 expression in prostate cancer (PCa) patients. The results have shown that low miR-199b-5p level and /or DDR1 positive expression were significantly correlated with the high Gleason score, distal metastases, and shorter overall survival in patients with PCa (all $P < 0.05$). In response to Cristóbal et al.'s concern, we therefore perform the univariate and multivariate Cox regression analyses to evaluate the survival impact of miR-199b-5p and DDR1 expression in PCa patients. As shown in Table 1 of this letter, univariate analysis indicated that miR-199b-5p negative expression and DDR1 positive expression could predict shorter disease recurrence-free survival in PCa patients ($P = 0.043$ and $P = 0.004$, respectively), however, multivariate analysis demonstrated that DDR1 positive expression was an independent predictor for patients' disease recurrence-free survival ($P = 0.009$). Taken together, our results demonstrated that DDR1, as a direct functional target of miR-199b-5p, could serve as an independently prognostic marker of poor outcome in PCa.

DDR1 has previously been reported to regulate EMT in various tumors and via different signaling pathways. Recently, Azizi et al. [3] using immunocytochemistry and western blot showed that knockdown DDR1 with targeted siRNA, in contrast with the stimulation of DDR1 with collagen-I, caused decreased phosphorylation levels of Pyk2 and MKK7 signaling molecules that led to significantly attenuated EMT and migration in PCa DU-145 and LNcap-FGC cells. Scientifically and logically, our study used the loss-and gain-of-functions and rescue experiments to reveal that ectopic up-regulation of miR-199b-5p and DDR1 in both LNCaP and PC-3 cells could attenuate the suppressive effects of miR-199b-5p on cell migration, invasion and metastasis, the prelude of which is EMT, whereas DDR1 silencing by shRNA phenocopied the inhibitive roles of miR-199b-5p. Furthermore, we observed that DDR1-overexpressed LNCaP and PC-3 cells presented EMT-associated phenotype and molecular markers, along with the activation of p-ERK protein, whereas ectopic supplementation of miR-199b-5p or down-regulation of DDR1 could significantly repress these EMT-associated traits of PCa cells and p-ERK protein expression, both in vitro and in vivo. A recent study reported that DDR1 binding with collagen in renal cell carcinoma (RCC) could activate ERK signaling through phosphorylation to stabilize the prometastatic factors SNAIL1/2 proteins that led to RCC metastasis [4]. Activation of p-ERK pathway has been reported to induce metastasis and EMT in PCa [5, 6]. Collectively, we therefore believe that miR-199b-5p/DDR1 regulated EMT via activating p-ERK signaling in PCa.

We agree with Cristóbal et al.'s opinion that the regulation of EMT and ERK by miR-199b could be involving a more complex signaling network that includes more actors than DDR1. SET might be another factor that plays this modulating role. As stated in the comment, Cristóbal's team and other investigators found that SET,

Table 1. Prognostic value of miR-199b-5p and DDR1 expression for disease recurrence-free survival of prostate cancer patients in univariate and multivariate analysis by Cox regression.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Age	1.439	0.652–3.176	0.367	–	–	–
PSA level at diagnosis	1.01	0.99–1.02	0.52	–	–	–
Gleason score	3.859	1.088–13.68	0.045	8.639	1.155–64.637	0.036
PT stage	2.2	1.19–4.08	0.012	0.54	0.27–1.07	0.08
Lymph node involvement	1.97	0.25–15.74	0.53	–	–	–
miR-199b-5p (−)	0.428	0.188–0.973	0.043	0.453	0.2–1.026	0.058
DDR1 (+)	5.63	1.74–18.26	0.004	4.93	1.5–16.22	0.009

PSA prostate-specific antigen, *PT* pathological tumor, *HR* harzard ratio, *CI* confidence interval, *DDR1* discoid domain receptor 1.

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as the direct target of miR-199b played oncogenic roles in colorectal cancer and PCa, and could promote EMT in pancreatic cancer. Therefore, we are also very interested in elucidating the molecular mechanism of miR-199a/b-DDR1-SET/PP2A in PCa in the forthcoming studies.

As described above, our results in the study demonstrated that miR-199b-5p-DDR1-ERK signaling axis regulated EMT in PCa at the cellular and animal levels. We have not analyzed the changes of expression levels of EMT markers after ectopic modulation of miR-199b or DDR1 in PCa cells treated by using an ERK inhibitor, however, we believe that this does not affect the main study findings. Indeed, as we have mentioned in the Discussion section of paper, the regulatory mechanisms and functional roles of miR-199b/DDR1 activating ERK-mediated EMT signaling pathway in PCa need further investigation.

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DATA AVAILABILITY

Not applicable.

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AUTHOR CONTRIBUTIONS

SZ drafted the first version of the letter. ZZ critically reviewed and edited the manuscript. All authors have read and approved the final version of the manuscript. ZZ and SZ have contributed equally to this work.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT TO PUBLISH

Not applicable.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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