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Title: Differential microRNA expression in epithelial cell populations from human prostate: its relevance to treatment resistance in prostate cancer

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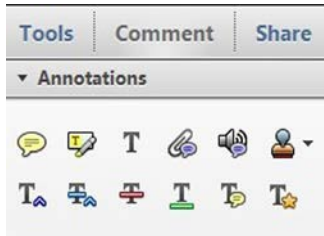


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# Differential microRNA expression in epithelial cell populations from human prostate: its relevance to treatment resistance in prostate cancer

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*Provenance:* This is an invited Correspondence commissioned by Section Editor Hongcheng Zhu (Department of Radiation Oncology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China).

*Response to:* Giridhar KV, Kohli M, Wang L. Is microRNA expression profile in prostate cancer dependent on clinicopathologic stage or cell subtype? *Transl Cancer Res* 2016;5:S1139-S1141.

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1 Since prostate cancer is a heterogeneous disease both  
2 between patients and at the cellular level, within  
3 patients, all population studies result in a median value  
4 for whatever parameter is being measured. Genome  
5 sequencing (and phenotyping) have contributed  
6 massively to the resolution of inter-patient heterogeneity,  
7 defining patient groups according to treatment response,  
8 clinical grade and of course genomic fingerprint, but  
9 nevertheless statistical outliers persist. Is this because  
10 cancer is inherently heterogeneous, with several pathways  
11 capable of resulting in a final aggressively growing and  
12 invasive phenotype, or is it because sophisticated studies  
13 are still being carried out on heterogeneous mixtures  
14 of cells?

15 In our recent study (1) as discussed by Giridhar *et al.* (2)  
16 in this journal, we adopted the same approach as we  
17 had many years ago for mRNA phenotypes (2), but now  
18 deliberately set out to test the hypothesis that the apparent  
19 non-concordance of the multiple miRNA studies in prostate  
20 cancer tissues was a direct result of heterogeneous cell  
21 mixtures. In fact little account was taken in earlier studies  
22 of e.g., stromal involvement, when extracting whole tissue  
23 biopsies, even after tissue microdissection. Did this mean  
24 that all previous genomic studies were wrong? I do not think  
25 so, except that the significant data may be hidden within a  
26 mixture, and as specific phenotypes for different cell types  
27 are determined, new software tools can presumably extract

significance. 28

We do agree with the authors of the commentary that  
the necessity to culture our cells for even a short time  
can skew the data, but since we are comparing different  
lesion types ALL of which are cultured, then we hope that  
culture artefacts will be in common and eliminated by our  
analysis. As we have shown previously (3), the expression  
levels of some mRNAs for secretory proteins in luminal  
cells are up to three orders of magnitude higher than in  
basal cells—implying that even a 1% contamination will  
result in a ten-fold higher expression. The need for careful  
fractionation methodology—and the sacrifice of yield for  
homogeneity cannot be overemphasised as mentioned  
further by Giridhar *et al.* (2). Ideally, fractionation should  
be simple and multifactorial (as we have demonstrated),  
but there is no golden rule, apart from a need to identify  
cell populations based on several independent factors, a  
lesson learned by haematologists long before epithelial  
biologists. 29  
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Such whole genome comparisons often result in a  
number of subsequent focussed analyses, and the Rane  
*et al.* study (1) is no exception. In a more recent paper (4)  
we described in more detail the analysis algorithm, which  
related miRNA expression to mRNA expression in the  
same cell populations. From this, we identified “radiation  
response” as a dominant gene ontology term—and in  
particular the role of the miR-99a/100 family. Whereas miR-  
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548c-3 showed striking effects on the stem-like phenotype of prostate epithelial cells, miR-99a/100 did not—mRNA suppressed by miR-99a/100 did however contribute to radiation sensitivity in both established prostate cell lines and primary cells from human prostates (5). In the latter paper we showed that the most significant miR-99a/100 target genes encoded two SWI/SNF chromatin remodeling factors, *SMARCA5* and *SMARCD1*, whose role in chromatin condensation has been defined previously. Manipulation of SMARCA5/D1 expression by means other than miRNA also affected radiation resistance, implying that part of stemness and radiation resistance is the presence of highly condensed chromatin. This agreed with our earlier studies, using HDAC inhibitors to unwind chromatin in stem-like cells (6), which resulted in greater radio-sensitivity. Finally, and unexpectedly, we showed that the chromatin state could be manipulated by glucocorticoid (GC) levels, via regulation of SMARCs. For example, administration of GC receptor inhibitors was able to promote radio-sensitivity in SC in a similar manner to HDAC inhibitors. This would imply that clinical application of GC response inhibitors such as Mifepristone in combination with standard radiotherapy protocols should improve outcomes. However, as for many chemotherapies (e.g., docetaxel) when GC supplements are administered to improve patient wellbeing, this would seem to fly in the face of standard clinical practice.

Lastly and perhaps with most significance for the future, the increasing applicability of single cell genomics and transcriptomics is set to transform the study of intratumoral cell heterogeneity. There have already been a number of examples, published with both solid and liquid (blood borne) tumour cells. The analysis has confirmed the expected heterogeneity, but here there is also a risk. If the single cell analysis is carried out as an exercise to confirm preconceptions from whole tissue analysis, then it is likely to ignore certain cell types as experimental artefact, particularly when these cells are in low abundance. There may indeed be several cell phenotypes in a cancer with stem-like properties—but is it the most common which is the most invasive or treatment resistant? To detect the stem-like cells we have defined in prostate cancer, would require the sequencing of >1,000 cells from a random sample. Whilst this will be accessible using new barcoding technologies (7) to give an identity to each cell in a complex mixture, there is also a case for selection based not on phenotype, but rather on biological

properties, prior to sequencing. In most experiments >99% of cells in a prostate tumour are non-tumorigenic in immuno-compromised mice. If you eliminate the stem-like cells for example by blocking STAT3 signalling from an IL6 stimulus (8), then you prevent tumour induction. Unfortunately, current treatment strategies shrink existing cancers by treating the majority (non-tumour initiating) population. It probably does not matter what the genotype of the latter cells are, at 10× or even 100× sequencing coverage. To achieve longer lasting treatments both stem-like and replicating bulk tumour cell populations must be destroyed.

### Acknowledgements

None.

### Footnote

*Conflicts of Interest:* The author has no conflicts of interest to declare.

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