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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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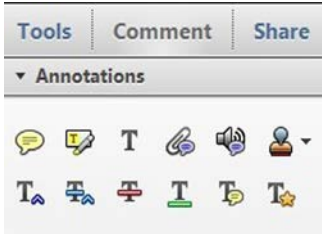


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Differential microRNA expression in epithelial cell populations from human prostate: its relevance to treatment resistance in prostate cancer

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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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