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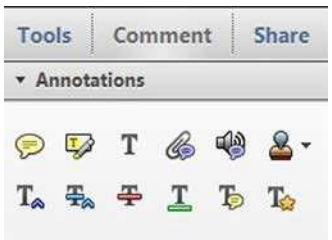

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Correspondence

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

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

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

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.

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-

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 SMARC A5/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.

References

1. Rane JK, Scaravilli M, Ylipää et al. MicroRNA Expression Profile of Primary Prostate Cancer Stem Cells as a Source of Biomarkers and Therapeutic Targets. Eur Urol 2015;67:7-10.
2. 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.
3. Birnie R, Bryce SD, Roome C, et al. Gene expression profiling of human prostate cancer stem cells reveals a pro-inflammatory phenotype and the importance of extracellular matrix interactions. Genome Biol 2008;9:R83.
4. Rane JK, Ylipää A, Adamson R, et al. Construction of therapeutically relevant human prostate epithelial fate map by utilising miRNA and mRNA microarray expression data. Br J Cancer 2015;113:611-5.
5. Rane JK, Erb HH, Nappo G, et al. Inhibition of the glucocorticoid receptor results in an enhanced miR-99a/100-mediated radiation response in stem-like cells from human prostate cancers. Oncotarget 2016;7:51965-80.
6. Frame FM, Pellacani D, Collins AT, et al. HDAC inhibitor confers radiosensitivity to prostate stem-like cells. Br J Cancer 2013;109:3023-33.
7. Macosko EZ, Basu A, Satija R, et al. Highly Parallel

Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets. Cell 2015;161:1202-14.
8. Kroon P, Berry PA, Stower MJ, et al. JAK-STAT blockade inhibits tumor initiation and clonogenic recovery of prostate cancer stem-like cells. Cancer Res 2013;73:5288-98.

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