Pathological Society Small Grants Scheme Report (grant number: 1129)

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Project title: Exploring Spatial Heterogeneity of microRNA Expression in Bladder Cancer

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Background

Non-muscle invasive bladder cancer (NMIBC) accounts for 75% of bladder cancer cases and is one of the most expensive cancers to manage due to the need for regular surveillance and repeat interventions with the aim of reducing progression to muscle invasive bladder cancer (MIBC)[1,2]. Previous studies have evaluated using molecular markers to predict progression and streamline care pathways to avoid this unfavourable event and reduce over treatment. One promising molecular approach is measuring microRNA expression. MicroRNAs (miRNA) are small non-coding RNAs which participate in the regulation of gene expression and have been implicated in bladder cancer development and progression [3,4]. Recently, spatial differences of genomic and transcriptomic profiles within a single tumour (intra-tumour heterogeneity; ITH) has been shown to affect the accuracy of gene expression and microRNA signatures [5,6]. However, this has not been evaluated in NMIBC.

Aims

1. Assess the level of ITH of microRNA expression in NMIBC
2. Determine if there is a relationship between microRNA ITH and progression in NMIBC

Results

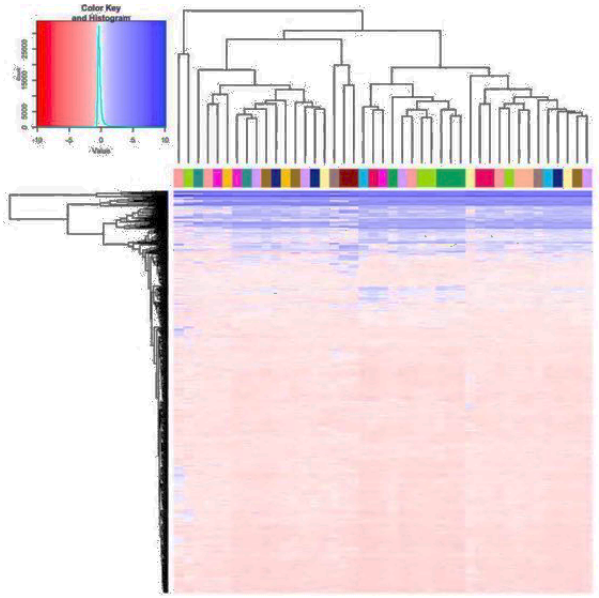
We used GeneChip miRNA 4.0 arrays to measure global microRNA expression in 15 cases of NMIBC, sampling at least two regions per case. The cohort was selected to include 9 cases with no progression to MIBC ('non-progressors') and 6 cases with progression to MIBC ('progressors'). The cases were balanced for clinical and pathological variables. Progression occurred after a median of 25 months in the progressor group. All samples were from index TURBT.

For each case we calculated two heterogeneity metrics: 1-pair wise Spearman correlation coefficient (SCC), and mean euclidean difference (MED). For both metrics a higher value indicates greater ITH. The median SCC in the two groups was 0.62 vs. 0.545 (non-progressors vs. progressors). The median MED was 46.56 vs. 43.4. Neither of these differences was statistically significant and there was marked variation in heterogeneity in both groups. Heterogeneity was visualised using hierarchical clustering and a heatmap of distances between individual tumour regions. Regions from the same tumour tended to cluster together, indicating greater inter- than intra-tumour heterogeneity (figure 1).

We used the coefficient of variation to measure ITH at the level of individual microRNAs as described previously [5]. Whilst the majority of microRNAs had low ITH in each individual case, some demonstrated consistently higher levels of ITH. Of these, miR148a and miR192 have previously been implicated in bladder cancer progression and further work is needed

to elucidate the biological significance of ITH in these transcripts. When we assessed ITH in the top percentile of genes with highest CV (representing those with greatest ITH) we observed no difference in our ITH metrics between progressors and non-progressors.

A.



B.

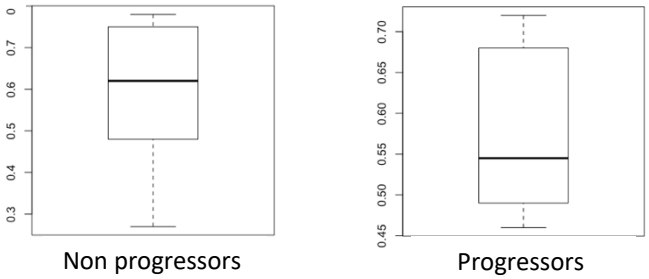


Figure 1. Intra-tumour heterogeneity in NMIBC. A. Hierarchical clustering of miRNA expression in all tumour regions. Each coloured bar represents a different case and demonstrates clustering of regions from each case. B. Median SCC values from progressors and non-progressors.

Summary

The level of microRNA expression ITH is low in NMIBC and there is no significant difference between progressors and non-progressors in our small cohort. This implies that ITH is unlikely to be significant in the progression of NMIBC, at least in the early stages of disease. Higher ITH is seen in some potentially biologically significant microRNAs.

Impact and future work

- Data generated in this project contributed towards two genomic medicine MSc projects.
- We aim to present the results of this project at a meeting of the Pathological Society
- Future research is directed at assessing temporal changes in ITH and further investigating the biological significance of microRNAs that showed higher levels of ITH.

We should like to thank the Pathological Society for supporting this project.

References:

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