Evaluation of Microarrays for Measuring CCP Gene Expression

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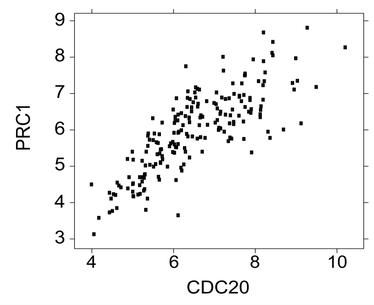
BACKGROUND

- The Cell Cycle Progression (CCP) score has been developed and validated to provide prognostic information in prostate cancer.[1]
- Precise CCP score calculation is dependent on the fact that individual CCP genes are highly correlated (Figure 1), allowing the average expression to estimate the true expression level.

Figure 1. CCP Gene Correlation of FF Tissue on microarray



- The amount of prognostic information derived from any biomarker is dependent on variation within the patient population.
- Our studies have exclusively used qRT-PCR to measure CCP gene expression.
- While qRT-PCR is generally considered the 'gold-standard' for measuring RNA expression levels, there are limitations in the number of specific targets.
- As prostate cancer biopsies provide very limited tissue, there is interest in RNA expression platforms that could simultaneously integrate CCP genes and other less characterized targets that might be clinically useful.
- Here we evaluate the ability of microarrays to measure CCP gene expression.

METHODS

- 1,636 radical prostatectomy (RP) samples submitted for commercial CCP testing (Myriad Genetic Laboratories) using qRT-PCR.
- The CCP score is the mean expression of 31 CCP genes normalized by 15 housekeeper genes.
- CCP scores were also generated from several publicly available microarray expression data sets[2-6] by averaging the intensities (RMA processed, base 2 log scale) of the 31 CCP genes.
- Expression data were evaluated by comparing range of CCP scores and pairwise correlations between CCP genes.

RESULTS

Table 1. Average CCP gene expression correlation

Cohort	Sample	Method	Correlation	SD
Nakagawa et al[2]	FFPE	Affymetrix	0.20	0.12
Karnes et al[3]	FFPE	Affymetrix	0.25	0.20
Klein et al[4]	FFPE	Affymetrix	0.17	0.13
Boormans et al[5]	Frozen	Affymetrix	0.58	0.46
Taylor et al.[6]	Frozen	Affymetrix	0.43	0.18
Commercial	FFPE	qRT-PCR	0.67	0.79

SD - standard deviation FFPE- formalin-fixed paraffin-embedded
NOTE: Affymetrix microarray chips, all samples were RP

Figure 2. CCP Gene correlation in the microarray data from Nakagawa et al. (top) compared to qRT-PCR (bottom).

Colors correspond to CCP gene correlation where a value of 1 (red) corresponds to complete correlation, 0 (white) corresponds to no correlation, and -1 (blue) corresponds to negative correlation.

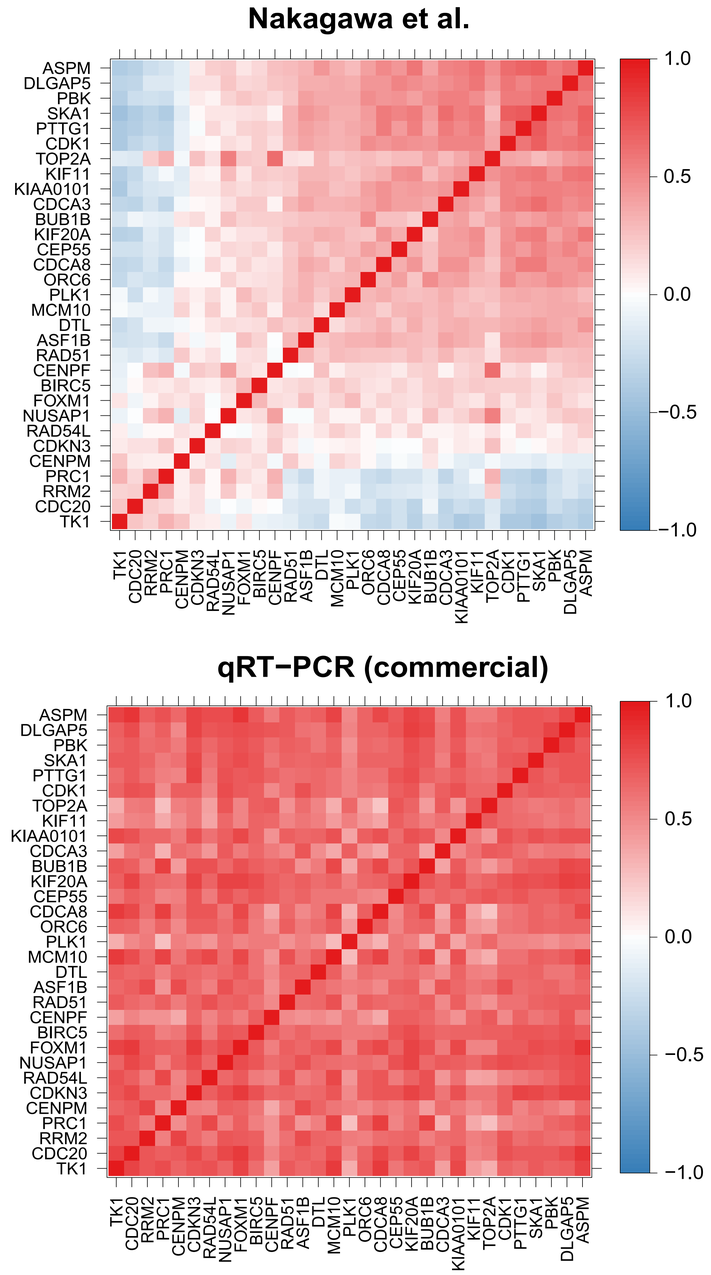
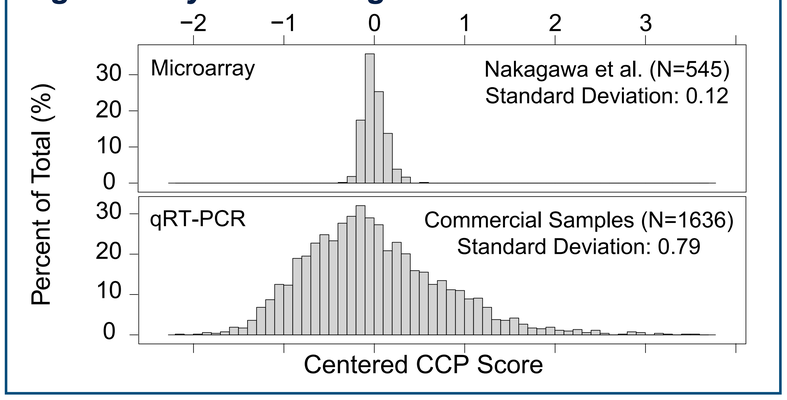


Figure 3. Dynamic Range of CCP Score



- The average pair-wise correlation of CCP gene expression was 0.67 in the commercial cohort (qRT-PCR), compared to only 0.17 to 0.58 in the publicly available cohorts (microarray) (Table 1, Figure 2).
- The highest correlation from the microarray data (0.58) was observed in the Boormans et al.[4] cohort; however, these data were generated from frozen tissue.
- The standard deviation of the CCP score distribution using qRT-PCR is 0.79 (Table 1, Figure 3).
- In contrast, the CCP score distribution is severely truncated in expression data derived from microarrays.

CONCLUSIONS

- Microarray determination of CCP expression correlates weakly with qRT-PCR measurements with reduction in both the normally observed pairwise correlations among CCP genes and in the range of CCP scores.
- This is especially true if RNA is derived from fixed tissue.
- Microarrays cannot accurately determine the CCP signature scores necessary for prediction of patient outcome.

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