Evaluation of Microarrays for Measuring CCP Gene Expression

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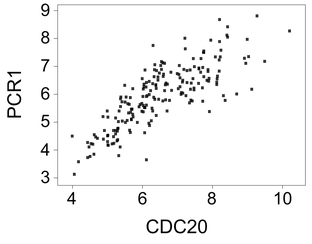
BACKGROUND

- The Cell Cycle Progression (CCP) score is validated to provide prognostic information in prostate cancer.[1]
- Most of the studies evaluating CCP gene expression have used qRT-PCR to measure expression levels, which is generally considered the 'gold-standard' for measuring RNA expression, but it is not a highly multiplexed platform.
- Because prostate tumor tissue is limited, there is interest in evaluating the ability of microarrays to measure CCP gene expression.

METHODS

- We first compared the characteristics of CCP scores from radical prostatectomy (RP) samples submitted for commercial testing (N=1,636 Myriad Genetic Laboratories), generated using qRT-PCR, with scores generated from several publicly available microarray expression data sets.[2-6]
- We then compared CCP scores generated by two different testing platforms using the same set of 39 contemporary RP samples: (1) CCP, Myriad Genetic Laboratories (qRT-PCR); (2) Cuzick CCP score, Decipher Grid (microarray).
- We evaluated the quality of CCP gene expression by:
  - Comparing pairwise gene correlation. This is expected to be high, because expression level is a measure of the average proliferation rate of the assayed tumor tissue (i.e., there is only one correct answer) (Figure 1).
  - Evaluating score variation, because the amount of prognostic information derived from any biomarker is dependent on variation within the patient population.
  - Comparing the correlation of proliferation scores between RNA expression platforms.

Figure 1. CCP Gene Correlation of FF Tissue on Microarray



RESULTS

- The average CCP gene pairwise correlation in the commercial RP cohort was 0.67 (Table 1; Figure 2).
- In contrast, the pairwise CCP gene correlations in the microarray studies were significantly lower, ranging from 0.17 (Klein[4]) to 0.58 (Boormans, which used frozen tissue[5]) (Table 1; Figure 2).
- The centered CCP score in our commercial cohort ranged from -2 to 3 (base 2 log scale), with a standard deviation (SD) of 0.79 (Figure 3).
- In contrast, the centered CCP score range in microarray studies was highly truncated, with SDs ranging from 0.12 (Nakagawa[2]) to 0.46 (Boormans[5]) (Figure 3).
- Finally, we used contemporary clinical samples to compare array- and qRT-PCR-based proliferation scores generated from the same FFPE tissue blocks (Figure 4A and B).
  - The between-platform correlation was only 0.60.
  - Further, the range of the array-based score (Cuzick score) was severely truncated compared to scores from qRT-PCR (total range 0.6 vs. 3.5).

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 3. Dynamic Range of CCP Score

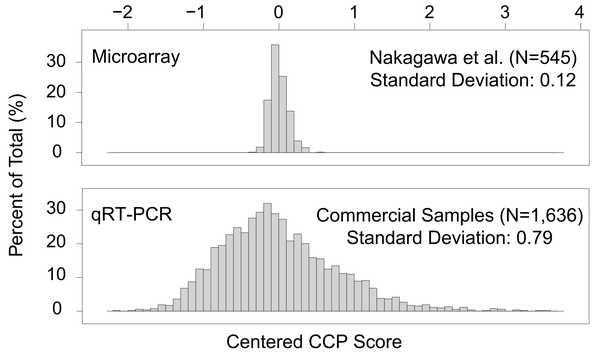


Figure 4. Correlation of Myriad and GenomeDx Cuzick Scores Arkansas Urology Cohort (N=39)

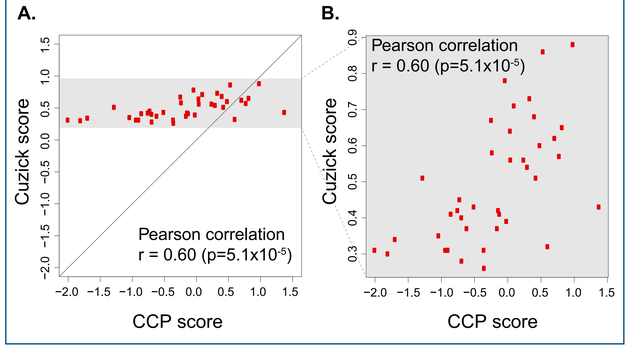


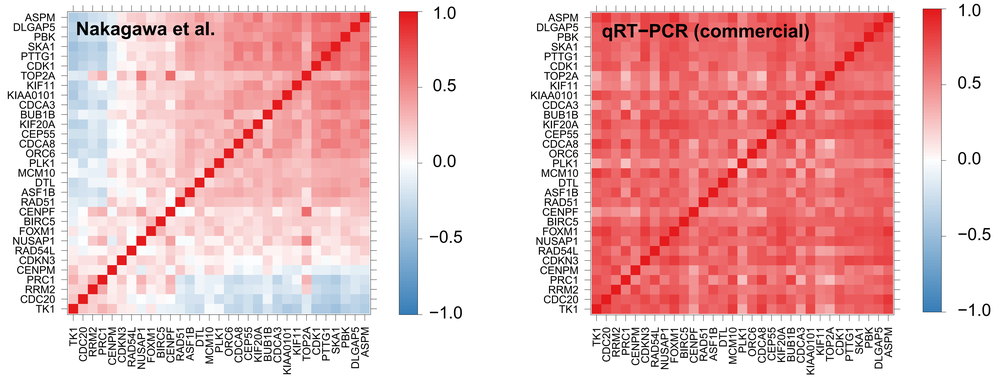
Figure 2. CCP Gene Correlation in Microarray Data from Nakagawa et al (left) Compared to qRT-PCR (right)

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



CONCLUSIONS

- Expression of CCP genes as determined by microarrays compared poorly with expression as measured by qRT-PCR.
- Using either contemporary or archival samples, we observed a reduction in pairwise correlations among CCP genes, limited CCP score range, and poor score correlation between platforms.
- As a result, microarray-generated CCP scores should not be assumed to be a valid surrogate for qRT-PCR generated scores for prediction of patient outcome.

REFERENCES

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