Supplementary methods

City of Hope patient characteristics, tissue samples, and mRNA profiling

This study was approved by the City of Hope (COH) Institutional Review Board (IRB07244). Patients with PC that were treated with RP between 1998 and 2013 at COH National Medical Center were selected based on age at diagnosis and tissue availability; in addition to 49 patients with Gleason score of 7 (3+4) reported previously[1], a total of 37 patients with Gleason score of 6 and 33 patients with Gleason score of 8–10 (Gleason 8+) were included in this study. Patient characteristics are shown in **Table 1**; a total of 61 men diagnosed between ages 71–75 years (old) and 58 men diagnosed between ages 38–50 years (young) were used to identify age-related differentially-expressed genes (DEGs) for developing a gene expression classifier to predict metastasis following RP. Tissue processing and mRNA profiling were performed as previously described[1]. Briefly, a genitourinary pathologist reviewed hematoxylin and eosin (H & E) slides and circled representative tumor or benign tissue on H&E slides from prostatectomy. Punch cores from the circled areas were obtained from the formalin-fixed paraffin-embedded blocks. RNA was extracted using RecoverAll™ Total Nucleic Acid Isolation kit (Life Technology Inc.). RNA quality was assessed using DV200 metrics, the percentage of RNA fragment size greater than 200 nucleotides, generated by running Nano chips from Agilent. RNA samples with DV200 value > 0.3 were selected for mRNA expression analysis. mRNA expression profiling of 29,000 genes (including some miRNA and LincRNA) in the human genome was generated using the Illumina Human Whole-Genome DASL (cDNA-mediated annealing, selection, extension, and ligation) HT Assay at the Genomics Core at the Case Western Reserve University. Tissue samples from patients (n = 70) with low (6) and high (8–10) Gleason score were processed and run at the same time on the cDNA-mediated Annealing, Selection, extension and Ligation (DASL) arrays (Illumina, Inc). Gene expression data for tissue samples from patients (n = 49) with Gleason score of 7 reported previously were also generated using the same Illumina DASL chips[1]. Follow-up data for the 119 COH patients were abstracted from medical records and the COH cancer registry.

Identification of age-related DEGs

DEGs [absolute fold change > 1.5 and false discovery rate (FDR) < 0.05] were identified from the DASL expression data using a mixed linear model implemented in the limma R Package[2]. In the model, sample type with two levels (tumor and benign), age group with two levels (young and old), and Gleason score with two levels [low (Gleason score 6) versus high (Gleason score of 8–10)] were categorical variables with fixed effects, and patient ID was treated as a random effect. From this model, DEGs were extracted from six comparisons: 1) Gleason-score-6 tumor versus matched benign prostatic tissue in young patient group; 2) Gleason-score-6 tumor versus matched benign prostatic tissue in old patient group; 3) Gleason-score-8 tumor versus matched benign prostatic tissue in young patient group; 4) Gleason-score-8 tumor versus matched benign prostatic tissue in old patient group; 5) Gleason-score-6 tumor versus Gleason-score-8 tumor in young patient group; and 6) Gleason-score-6 tumor versus Gleason-score-8 tumor in old patient group. the Benjamin-Hochberg (BH) method[3] was used to correct for multiple testing. In a previous study[1], we identified genes expressed differentially between Gleason-score-7 tumor and matched benign tissue in young and old patient groups. DEGs identified from these eight comparisons were considered as primary candidate genes for developing a genomic classifier to predict metastasis following radical prostatectomy (RP) [**Supplementary Figure 1**]. Ingenuity pathway analysis (IPA) of DEGs was used to predict directional biological effects (pathway involvement, cellular function, and disease association) of DEGs.

iPAM classifier development and validation

Gene expression data from primary tumors from RP in five data sets from the Decipher Genomic Resource Information Database (GRID, Decipher Biosciences, San Diego, CA) were used to develop and validate a new genomic classifier. Clinicopathological characteristics of the 1232 patients in the five datasets are shown

in **Supplementary Table 1**; none of the patients had regional or distant metastatic disease at the time of RP. Gene expression data were generated using the Affymetrix Human Exon 1.0 ST Gene Chips array and normalized as one combined data frame (46,050 genes in rows and 1232 patients in columns). A diagram of our study design is shown in **Supplementary Figure 1**.

The Mayo Clinic discovery cohort (MC I)[4] of 545 patients was used to develop the genomic classifier. In this data set, 212 patients developed early regional or distant metastasis (confirmed by bone or CT scan) within five years of biochemical relapse (BCR). This cohort was previously used to develop the Decipher classifier based on the random forest method[4]. Gene expression data from the MC I cohort for the age-related DEGs identified from the COH samples were extracted. To further reduce the DEG list, a two-sample t-test was used to only include genes differentially expressed between patients with (n = 212) and without (n = 333) metastasis. After determining the list, the 545 patients from the MC I cohort were randomly assigned into a training data set (140 with and 222 without metastasis) and a test dataset (72 with metastasis and 111 without metastasis). In order to select the optimal number of age-related DEGs that were most informative in predicting the development of metastatic prostate cancer (CaP), an improved Prediction Analysis of Microarray (iPAM) method[5-7] was applied to the training data to remove DEGs irrelevant to metastasis prediction based on minimizing the 10-fold cross-validated error rate. Specifically, the Adaptive Hierarchically Penalized Nearest Shrunken Centroid algorithm[6] was used to enable different amount of shrinkage for each variable (gene) in the process of variable selection. These iPAM-selected DEGs were assembled into an iPAM classifier by fitting a logistic regression model on the 362 samples in the training set. The iPAM classifier was then used to predict metastasis for the four independent validation data sets from the Decipher Biosciences [**Supplementary Table 1]**. The training data also were used to develop a clinical classifier including six clinical variables [pathological Gleason score (GS), preoperative PSA, seminal vesicle invasion (SVI), extra-capsular extension (ECE), lymph node invasion (LNI), and surgical

margin (SM)] [**Supplementary Table 1**] as predictors in a logistic regression model to predict metastasis. An integrated classifier was also constructed by combining informative DEGs selected by the iPAM method and the six clinical variables as predictors in a logistic regression model.

Estimation of cell-type proportion in tissue microenvironment

xCell[8] was used to estimate the enrichment score of individual cell types (21 lymphoid, 13 myeloid, 14 stromal, 9 stem, and 7 epithelial cell types) for each tissue sample using genome-wide gene expression data (> 9000 specific genes). xCell also generated an immune score representing the overall abundance of immune cells for each sample by summing the enrichment scores from all immune cell types. The DASL gene expression data from 238 COH tissue samples (119 tumor-normal pairs) and Affymetrix gene expression data from 1232 primary tumor samples from the decipher GRID were analyzed separately by the xCell method. Cell-type proportion in tissue microenvironment estimated by xCell method is a rank-based enrichment score. Non-parametric analysis of variance (ANOVA) (confidence interval and p-values generated by percentile bootstrap), implemented in the "Rallfun-v35" R codes from Dr. Wilcox[9], was used to test median differences in immune scores between sample groups classified by factors of sample type (tumor, normal), metastasis status (yes, no), and age group (old, young, middle-age).