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Distinguishing benign renal tumors with an Oncocytic Gene Expression (ONEX) Classifier

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Abstract

Renal oncocytoma (RO) accounts for 5% of renal cancers and generally behaves as a benign tumor with favorable long-term prognosis. It is difficult to confidently distinguish between benign RO and other renal malignancies, particularly from chromophobe renal cell carcinoma (chRCC). Therefore, RO is often managed aggressively with surgery. We sought to identify molecular biomarkers to distinguish RO from chRCC and other malignant renal cancer mimics. In a 44-patient discovery cohort, we identified a significant differential abundance of 9 genes in RO

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relative to chRCC. These genes were used to train a classifier to distinguish RO from chRCC in an independent 57-patient cohort. The trained classifier was then validated in 5 independent cohorts comprising 89 total patients. This 9 gene classifier trained on the basis of differential gene expression showed 93% sensitivity and 98% specificity for distinguishing RO from chRCC across the pooled validation cohorts, with a c-statistic of 0.978. This tool may be a useful adjunct to other diagnostic modalities to decrease the diagnostic and management uncertainty associated with small renal masses and enable clinicians to more confidently recommend less-aggressive management for some tumors.

Patient summary:

Renal oncocytoma is generally a benign form of kidney cancer that does not necessarily require surgical removal. However, it is difficult to distinguish renal oncocytoma from other more aggressive forms of kidney cancer, so it is most commonly treated with surgery. We built a classification tool based on the RNA levels of 9 genes that may help avoid these surgeries by reliably distinguishing renal oncocytoma from other forms of kidney cancer.

Keywords

molecular biomarkers; renal oncocytoma; chromophobe renal cell carcinoma; RNA expression; tumor classification

Renal oncocytoma (RO) is a benign tumor that is thought to arise from a progenitor cell in the distal convoluted tubule or intercalated cells of the collecting duct (1). RO represents about 75% of resected benign renal tumors and ~5% of all renal tumors (1) (2). The optimal management of RO has not been systematically studied, but even large ROs are rarely locally invasive (3). If RO could be reliably identified preoperatively, there would be a very low risk associated with active surveillance (4).

chRCC represents ~5% of all malignant renal neoplasms and originates from the same region of the nephron as RO (5). When chRCC metastasizes, treatment options are limited, leading most patients to die from their disease (6). There is no established imaging or molecular biomarker that can reliably distinguish RO from chrRCC prior to treatment. Apart from chRCC, other so-called oncocytic neoplasms may be differentiated from RO based on histology and immunohistochemical markers. For instance, oncocytic papillary RCC is distinguished by careful histological examination for papillary architecture and clear cell RCC with oncocytic features can be identified by characteristic immunohistochemical markers (positivity for CD10 and CA-9, negative for CK7 and high-molecular-weight keratin). As a result of the difficulty distinguishing RO from chRCC, most patients with a small renal mass consistent with RO proceed to surgery (7). This leads to unnecessary costs and complications in patients with RO, including a mortality rate of 0.2% and over $150 million in annual inpatient hospital charges in the United States (8). As the incidence of small renal masses has tripled over the past three decades, more ROs are being seen clinically and managed most commonly *via* partial nephrectomy (8).

With the significant burden of this benign disease in mind, we sought to identify a molecular biomarker that could reliably distinguish between oncocytoma and chromophobe RCC. We hypothesized that a classifier could be developed to distinguish RO and chRCC based on mRNA abundance. If operationalized, a classifier could be clinically implemented to distinguish between RO and chRCC from limited tissue qualities obtained via a renal mass biopsy to aid pre-treatment decision making. Our approach employed RNA profiling in discovery, training, and validation cohorts to reliably distinguish RO and chRCC based on transcript-level gene expression (Supplemental Methods) [Figure S1].

In a 44 patient discovery cohort (17 RO, 27 chRCC) (9), we found that the genes ADAP1, SDCBP2, HOOK2, BAIAP3 and SPINT1 were significantly up-regulated in chRCC while ITGB3, MINOS1-NBL1 and ASB1 were significantly up-regulated in RO (all genes with FDR < 0.1%, Supplemental Table S1) [Figures 1B, 1C, S1]. Given that these genes are significantly differentially expressed in these tumors, we evaluated their differential abundance in a separate 57-patient training cohort from Yale New Haven Hospital (30 RO, 27 chRCC). All 9 genes' directionality and significance in the training cohort was concordant with those in the discovery cohort: ADAP1, SDCBP2, HOOK2, BAIAP3 and SPINT1 were significantly up-regulated in chRCC while ITGB3, MINOS1-NBL1 and ASB1 were significantly up-regulated in RO (all FDR < 0.1%, Supplemental Table S1) [Figures 1C, 1D].

The functions of these genes are described in Supplemental Table S2. There were no high-confidence protein-protein interactions amongst them in the STRING database. The genes were enriched for phospholipid/phosphatidylinositol bisphosphate binding (FDR = 0.0401). The general functional independence of these 9 genes suggests that their abundances may act as relatively independent predictors of tumor class.

Because all 9 genes from the discovery cohort replicated in both their direction and significance in the training cohort, we then used the larger training cohort to train a classifier for RO *vs.* chRCC on the basis of the expression of these 9 genes. The classifier was trained using the method of Tibshirani *et al.* (10) which uses shrunken centroids to classify patients on the basis of molecular profiles. mRNA abundances were normalized per-sample by the summed expression of a set of housekeeping genes (see Supplemental Methods: Training Cohort) [Figure 1E].

The trained 9-gene classifier performed well in 10-fold cross-validation with 3 classification errors across the 57 patients (1 false positive RO classified as chRCC, 2 false negative chRCCs classified as RO) [Figure 1G]. Cross-validation fold-assignments, as well as classification probabilities in cross-validation for the training cohort, are listed on a per-sample basis in Supplemental Table S3. The sensitivity for prediction of cancer was thus 93% with a specificity of 97% in cross-validation. The receiver operating characteristic (ROC) curve in cross-validation on the training cohort also reflects its accuracy, with a c-statistic of 0.986 [Figure 1H].

Given the relatively strong contribution of certain genes to classification accuracy, this level of performance was sustained with just the 6 most performant genes included (genes

in order of descending centroid distance units as a proxy of classification performance: SPINT1, SDCBP2, ASB1, HOOK2, ITGB3, and BAIAP3, ADAP1, NBL1, MINOS1). However, performance decreased as fewer genes were included in classification. The upregulation of SPINT1 was the most suggestive of chRCC, while the upregulation of ASB1 was the most suggestive of RO classification [Figure 1F].

Given the strong performance of the shrunken centroid based classifier on the training set and in cross-validation, we evaluated its performance on five validation cohorts with balanced numbers of RO and chRCC samples (See Supplemental Methods: Validation Cohorts, Supplemental Table S4) [Figures 2A-E]. These validation cohorts comprised publicly available microarray data from independent institutions and study groups deposited in the GEO repository. Following housekeeping gene normalization, each dataset was separately classified using the pre-trained centroid classifier. The validation datasets were then combined sample-wise to generate a final validation cohort (45 RO, 44 chRCC) [Figure 2G].

Classification probabilities on a per-sample basis for each of the five validation cohorts are listed separately in Supplemental Table S5. The sensitivity and specificity of the classifier was robust across each of the validation cohorts, as listed in the legend for Figures 2A-E. The C-statistic in each validation cohort was ≥ 0.97. Overall, the classifier was 93% sensitive and 98% specific across all samples in the combined validation cohort, with a C-statistic of 0.978 [Figures 2F, 2H].Intratumoral heterogeneity may influence tumor classification when used on biopsy specimens. We therefore used tumor variability among patients to estimate the effect of intratumoral heterogeneity on classifier performance [Figure S2]. We found that even with simulated intratumoral heterogeneity up to 3× the observed inter-tumour heterogeneity, classifier performance remained strong (AUC=0.936).

Across the discovery, training and validation cohorts we assessed 190 separate patients (92 RO and 98 chRCC). Although prospective validation in even larger cohorts is warranted, the number of patients included in our study provides substantial confidence in the generalizability of this classification strategy. In clinical practice, gene expression testing is possible on core biopsy specimens and also on small numbers of cells obtainable with fine-needle aspirate. We conceive that such a high sensitivity gene expression assay could reduce costs and minimize some of the immediate and long-term morbidity from overtreatment of RO. A suggested algorithmic approach for application of the classifier is included as Figure S3.

The classifier would not need to be considered alone as other factors such as imaging characteristics, immunohistochemical markers, and patient factors could perhaps further increase its performance metrics. 99mTc-sestamibi SPECT/CT has recently gained attention as a non-invasive method to distinguish RO from chRCC. This method was reported to have 89% sensitivity and 67% specificity for distinguishing RO from chRCC [11]. Histopathologic identifiers that have been proposed to differentiate oncocytoma from chRCC include CK7, Hales colloidal iron, and EMA. The most promising of these markers is CK7, as chRCC generally shows diffuse strong staining for CK7 (although up to 18% of

chRCC samples may nonetheless be negative for CK7). These methods may complement our classifier in reaching a diagnosis.

In summary, the 9-gene classifier identified in this study demonstrated high sensitivity and specificity in differentiating RO and chRCC. As such, it can be used to decrease diagnostic and management uncertainty associated with small renal masses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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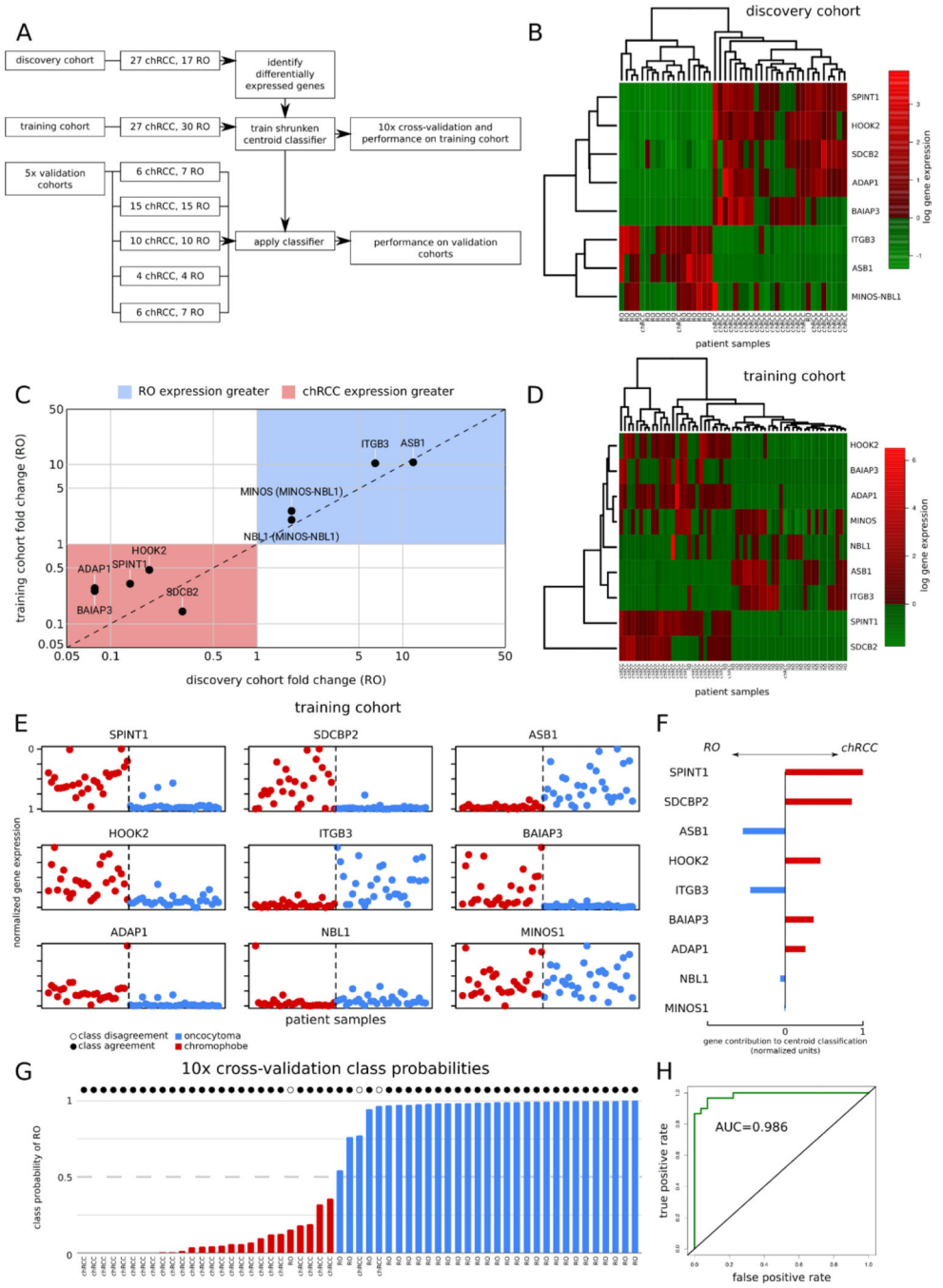

Figure 1: Discovery and Training Cohorts

A. *Methods overview.* The discovery cohort was used to identify genes with differential expression in RO vs. chRCC. This differential expression was then confirmed in a separate training cohort before using the training cohort to develop a shrunken centroid classifier for RO vs. chRCC. The pre-trained algorithm was then applied to five unique validation cohorts to quantify the accuracy of the classifier on novel data sets. B. *Discovery cohort expression heatmap*. The expression of the 9 genes identified as differentially expressed in the discovery cohort quantified in log10 normalized expression units. C. *Discovery and training cohort*

gene fold-changes. The fold-changes for the 9 genes in the discovery cohort *vs.* the training cohort on a log-scale (fold-changes for RO relative to chRCC). D. *Training cohort expression heatmap*. The expression of the 9 genes identified as differentially expressed in the discovery cohort quantified in log10 normalized expression units. E. *Training cohort gene expression.* Normalized gene expression in the training cohort for each of the 9 genes from the discovery cohort. Each point represents one patient sample in the training cohort. F. *Gene contributions to classification.* The relative contribution of each gene to the shrunken centroid classifier -- the distance between centroids -- normalized with respect to the gene that provides greatest the separation of classes (SPINT1). G. *Training cohort sample class predictions from cross-validation.* Class probabilities from 10-fold cross-validation of the shrunken centroid classifier applied to the training cohort (merged class probabilities from each of the 10 folds). H. *ROC curve in cross-validation*. The performance of the shrunken centroid classifier on the training cohort in cross-validation visualized as an ROC curve (AUC=0.986).

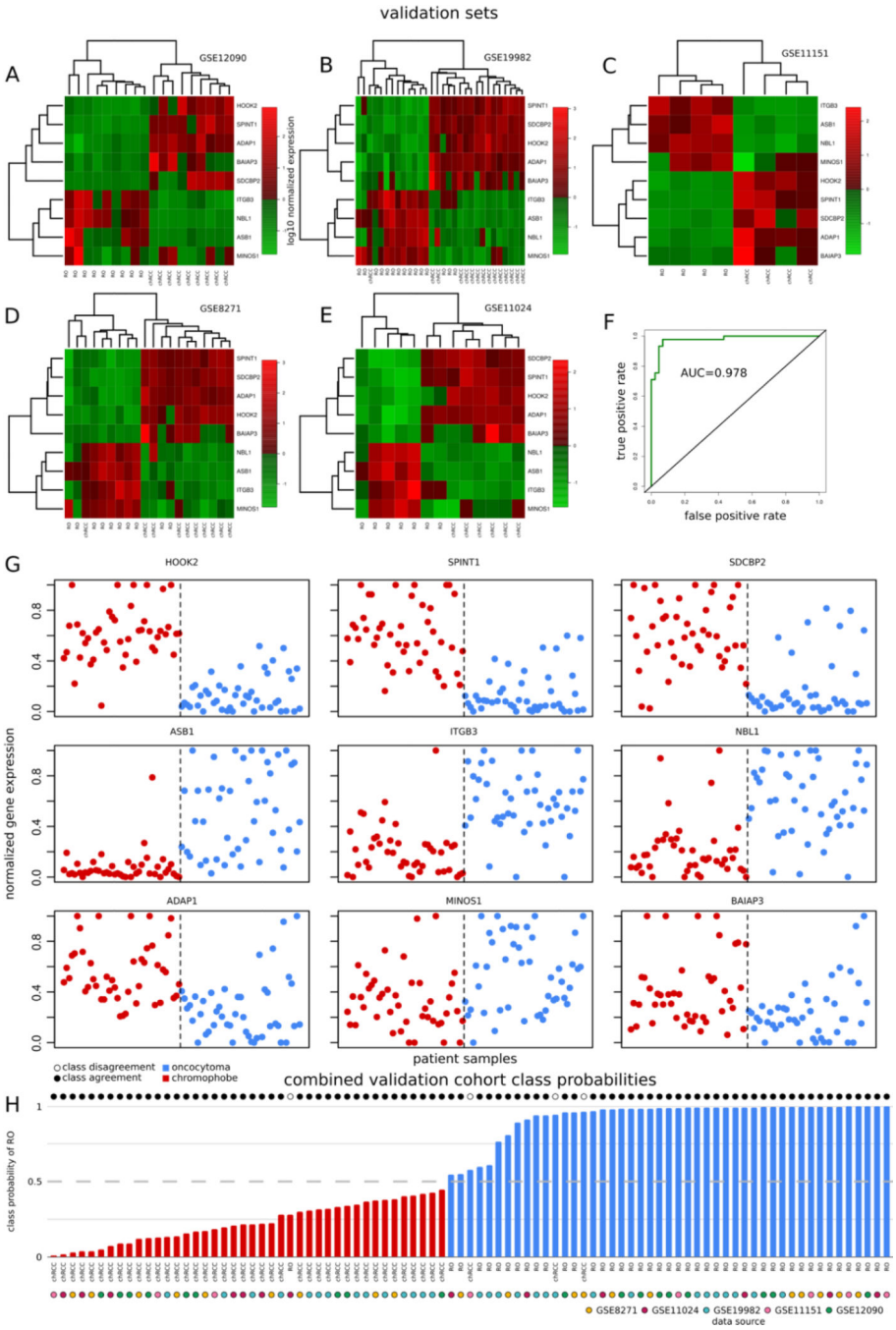

Figure 2: Validation of the 9 gene classifier

A-E: *Validation cohorts expression heatmaps*. The expression of the 9 genes quantified in log10 normalized expression units for each of the validation cohorts [A. *GSE12090.* (100% sensitivity, 100% specificity, C-statistic 1). B. *GSE19982.* (93% sensitivity, 100% specificity, C-statistic 0.973) C. *GSE11151.* (75% sensitivity, 100% specificity, C-statistic 1) D. *GSE8271.* (90% sensitivity, 100% specificity, C-statistic 0.97). E. *GSE11024.* (100% sensitivity, 86% specificity, C-statistic 1)]. F. *Combined validation cohorts ROC.* The performance of the shrunken centroid classifier on the combined validation cohorts

visualized as an ROC curve (AUC=0.978). G. *Combined validation cohorts gene expression.* Normalized gene expression in the combined validation cohort for each of the 9 genes from the discovery cohort. Each point represents one patient sample in the combined validation cohort. H. *Combined validation cohort sample class predictions.* Class probabilities from the application of the shrunken centroid classifier to the combined validation cohort.