Deep learning-based predictions of clear and eosinophilic phenotypes in clear cell renal cell carcinoma

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Abstract
We have recently shown that histological phenotypes focusing on clear and eosinophilic cytoplasm in clear cell renal cell carcinoma (ccRCC) correlated with prognosis and the response to angiogenesis inhibition and checkpoint blockade. This present study aims to develop an artificial intelligence (AI) model for predicting clear or eosinophilic phenotypes in ccRCC using the TCGA-KIRC dataset and to demonstrate if the predicted phenotypes correlate with pathological factors and gene signatures related to angiogenesis and cancer immunity. Prior to the development of the AI model, histological evaluation using hematoxylin and eosin (H&E) whole-slide images of the TCGA-KIRC cohort (n = 435) was performed by a urologic pathologist, similar to our previous studies, and successfully shown that clear and eosinophilic histological phenotypes correlated with clinicopathological characteristics, gene signatures, and prognosis, confirming that our diagnostic method based on the histological phenotypes by using the TCGA-KIRC cohort consisting of multi-institutional slides was valid. The AI model was developed as follows. First, the highest-grade area on each whole slide image was captured for image processing. Second, the selected regions were cropped into tiles to develop the AI model. Third, the AI was trained using transfer learning on a deep convolutional neural network, and clear or eosinophilic predictions were scaled as AI scores. Next, we verified the AI model using a validation cohort (n = 95). Finally, we evaluated the accuracy of the prognostic predictions of the AI model and revealed that the AI model detected clear and eosinophilic (mixed or predominantly eosinophilic) phenotypes with high prediction. The AI model stratified the patients’ outcomes in the TCGA-KIRC cohort (p = 0.008) and predicted eosinophilic phenotypes correlated with adverse clinicopathological characteristics and high immune-related gene signatures. In conclusion, an AI-based histologic subclassification accurately predicts clear or eosinophilic phenotypes in ccRCC allowing for consistently reproducible stratification for prognostic and therapeutic stratification.

Introduction
In advanced stages of clear cell renal cell carcinoma (ccRCC), systemic therapies utilizing tyrosine kinase or immune checkpoint inhibitors have been widely used [1]. Recent studies have focused on gene signatures related to angiogenesis and cancer immunity to determine the responsiveness to antiangiogenic or immune therapies [2, 3]. However, widespread gene expression analyses in routine clinical practice are compounded due to cost and technical considerations. Thus, characterizing clinically relevant phenotypes by hematoxylin and eosin (H&E) microscopy reflecting gene signatures associated with treatment responses is cost-effective and expeditious for more widespread clinical adoption.

Recently, our research group was the first to report that clear and eosinophilic histological phenotypes of ccRCC could serve as predictive markers for responsiveness to angiogenesis inhibitors or checkpoint blockades [4]. We believe that this distinction based on cytoplasmic tinctorial characteristics is important because it provides information on the patient's prognosis and treatment selection. The eosinophilic phenotype is associated with higher proliferation and lower differentiation and correlates with low angiogenesis and high immune-related gene signatures, as shown by Nilsson et al. [5].
Nevertheless, the assessment of clear or eosinophilic histological phenotypes is subjective, and the reproducibility of determining the phenotypes using multi-institutional slides has not been assessed. Therefore, the standardization and objectiveness of our method for identifying the histological phenotypes should be verified. Recent advances in artificial intelligence (AI) technology, including deep learning and neural networks [6] have contributed to improving the efficiency, accuracy, and consistency of histopathologic evaluations [7–10]. Thus, by training the AI algorithm to subtype ccRCC based on clear or eosinophilic cytoplasmic features, the model can indirectly predict the gene signatures relevant to therapy and prognosis.

The present study aims to develop an AI model based on transfer learning for predicting clear or eosinophilic phenotypes using The Cancer Genome Atlas (TCGA)-KIRC dataset [11] (development cohort, \( n = 435 \)) and correlate the AI model with pathological prognostic factors assessed by a pathologist, and gene signatures related to therapeutic responsiveness and prognosis [2, 12]. Next, we verified the AI model with histological features and gene expression using the validation cohort \( (n = 95) \). Finally, we evaluated the AI model’s prognostic prediction using the TCGA-KIRC cohort.

**Materials And Methods**

The study workflow is shown in Fig. 1. First, histological evaluations using whole-slide images from the development cohort (TCGA-KIRC) were performed by a urologic pathologist. The pathologist examined the histological phenotypes focused on clear and eosinophilic cytoplasmic features and whether these correlated with clinicopathological characteristics, gene signatures, and patient’ outcomes. Second, the highest-grade area of each whole slide image was extracted by two pathologists for image processing. Third, selected regions were cropped into tiles to develop the AI model. Fourth, the AI model was trained using transfer learning on a deep convolutional neural network, and clear or eosinophilic predictions were scaled as AI scores using a range of 0 to 1. Fifth, we verified the AI model using the validation cohort.

**Data sources of a development cohort**

The development cohort consisted of 435 cases of ccRCC from the TCGA-KIRC database \( (n = 435) \). One representative H&E whole slide image of each case was retrieved from the Digital Slide Archive [13]. Clinical variables, including gender, pathological stage, and survival information, were acquired from the database for every case [11].

**Histopathological evaluation**

The histological phenotypes such as clear, mixed, and eosinophilic were determined at the highest WHO/ISUP grade area in the same way as our previous studies [4, 14]. Briefly, the clear phenotype of ccRCC was defined as tumors composed of neoplastic cells with clear or pale cytoplasm, the eosinophilic phenotype as tumors composed of neoplastic cells with eosinophilic cytoplasm, and the mixed phenotype as tumors coexisting clear cells with eosinophilic cells if they were present in at least one high-power field of the highest-grade area. We also evaluated the cases for the following: the World Health
Organization/International Society of Urological Pathology (WHO/ISUP) grade, vascularity-based architectural category based on our previously published work [14], tumor-related necrosis [15], and three-tier morphologic immunophenotypes: desert, non-inflamed; excluded, peritumoral immune infiltration; and inflamed, intratumoral immune infiltration [14, 16]. In addition, all H&E images were assessed by a urologic pathologist (CO) blinded to clinical outcomes (Supplementary Figs. 1A-1C).

Gene expression analysis

TCGA RNA-sequencing data were downloaded as described previously [14]. The association of manually and automatically detected histological phenotypes with gene signatures related to therapeutic response and prognosis was examined using gene panels such as the IMmotion 150 gene signature panel [2], which consists of genes related to angiogenesis, immune and antigen presentation, and myeloid inflammation, and the ClearCode34 gene signature panel [12] consisting of genes related to hypoxia, angiogenesis, and fatty acid metabolism, which is upregulated in ccA classified ccRCC, and genes that regulate epithelial to mesenchymal transition (EMT), the cell cycle, and wound healing, which is upregulated in ccB classified ccRCC, were extracted from RNA-sequencing data from the TCGA cohort. Gene signature scores were calculated, and each gene score was normalized to the z-score across all patients and averaged to create signature scores for each patient (Supplementary Table 1) [17].

Image processing and transfer learning on a deep convolutional neural network

SVS-format whole-slide images available from the Digital Slide Archive [13] were converted into JPEG images and annotated by two pathologists (CO and RU) according to the previous method [4]. Areas of prominent hemorrhage, necrosis, fibrosis, and normal kidney tissue were not included in the extracted areas. Various colored images of clear or eosinophilic regions were selected and cropped into tiles sized 227 × 227 pixels. Our network parameters were initialized to the default parameters provided for the deep convolutional neural network, ALEXNET [18]. Then we fine-tuned the parameters of the last layer of networks on our data via backpropagation using two NVIDIA GeForce RTX 2080Ti graphic processing units. The loss of function was defined as the cross-entropy between predicted probabilities and true class labels. The weights were trained by stochastic gradient descent with momentum (SGDM) optimization with a learning rate of 0.001 and momentum of 0.9. Seventy percent of tiles consisting of 3,904 clear and 16,584 eosinophilic regions were used in training the transfer learning, and the remaining 30% were used for validation. After developing the AI model, we applied the model to the annotated regions of 435 cases derived from the TCGA-KIRC cohort. AI scores were scaled from 0 to 1, and class activation mappings [19, 20] of clear or eosinophilic predictions were visualized as cold (blue) and warm (red) colors, respectively.

Data sources of a validation cohort

To evaluate whether our AI model correlated with the histological features assessed by a pathologist and gene signatures, 95 samples consisting of clear (n = 30), mixed (n = 48), and eosinophilic (n = 17) phenotypes were extracted from ccRCC patients who underwent nephrectomy at the Kansai Medical
University Hospital [4]. We explored the RNA expression corresponding to the histological phenotype from tissue microarrays (TMAs) from two-millimeter cores of formalin-fixed, paraffin-embedded (FFPE) blocks. After removing three-micrometer slices from FFPE-TMA blocks for histological assessment of H&E-stained slides, the TMA cores were used for mRNA isolation using ReliaPrep FFPE Total RNA Mini-prep System (Promega, Madison, Wisconsin, USA) as previously described [4]. A custom NanoString panel from the IMmotion 150 [2] and ClearCode34 [12] panels were used as previously described [4]. H&E-stained TMA slides were scanned by a Philips IntelliSite Pathology Solution (Philips, Best, the Netherlands) to verify our AI model. This study was approved by the Institutional Review Board (No. 2018109 and No. 2020222).

**Statistical analysis**

Statistical analyses were performed using EZR version 1.54 (Saitama Medical Center, Jichi, Japan) [21]. All continuous data are shown as median-valued and interquartile ranges (IQRs). A Chi-squared test for categorical variables and a Mann-Whitney U test for non-parametric variables was used to evaluate the statistical significance among the two groups. The predictive accuracies of clear or mixed/eosinophilic phenotypes were assessed by a pathologist, and the AI score assessed by transfer learning was analyzed using the area under the curve (AUC). Overall survival was defined as the time from surgery to any cause of death in the TCGA cohort. Overall survival was assessed by the Kaplan-Meier method with log-rank tests and the Cox proportional hazards model. Harrell's concordance index (c-index) was used to evaluate the predictive accuracy of Cox models. For all analyses, significance was determined as a p-value less than 0.05.

**Results**

**Clinicopathological characteristics of the development cohort**

Clinicopathological characteristics and outcomes of 435 patients in the TCGA-KIRC cohort are summarized in Table 1. The rate of pathological prognostic factors such as TNM stage III or IV, WHO/ISUP grade 3 or 4, and the presence of necrosis was seen in 172 (39.6%), 220 (50.5%), and 64 (14.7%) patients, respectively. Of the 435 patients with ccRCC, 144 (33.1%) died during the median follow-up period of 1191 days (IQR, 556.5-1912.5). Histological phenotypes assessed by a pathologist were 162 (37.2%) patients with the clear phenotype, 230 (52.9%) with the mixed phenotype, and 43 (9.9%) with the eosinophilic phenotype.
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Gender, n (%)</td>
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<td>Female</td>
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<tr>
<td>Male</td>
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<td>TNM stage, n (%)</td>
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<td>2</td>
<td>186 (42.8)</td>
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<tr>
<td>3</td>
<td>155 (35.6)</td>
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<td>4</td>
<td>65 (14.9)</td>
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<tr>
<td>Necrosis, n (%)</td>
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<td>Histological phenotype, n (%)</td>
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<tr>
<td>Clear</td>
<td>162 (37.2)</td>
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<tr>
<td>Mixed</td>
<td>230 (52.9)</td>
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<tr>
<td>Eosinophilic</td>
<td>43 (9.9)</td>
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<tr>
<td>Vascularity-based architectural classification, n (%)</td>
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<tr>
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<td>182 (41.8)</td>
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<td>Immunophenotype, n (%)</td>
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Variables

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<td>Inflamed</td>
<td>155 (35.6)</td>
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<tr>
<td>Overall mortality, n (%)</td>
<td>144 (33.1)</td>
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</tbody>
</table>


cCRCC, clear cell renal cell carcinoma; WHO, World Health Organization; ISUP, International Society of Urological Pathology

Association of clear or eosinophilic histological phenotypes assessed by a pathologist with associated clinicopathological characteristics and gene signatures in the development cohort

Representative H&E-stained images of clear, mixed, and eosinophilic histological phenotypes are shown in Fig. 2A. The survival curve of the TCGA cohort stratified by histological phenotypes and survival analysis showed a five-year overall survival rate of 59.0% (hazard ratio [HR], 2.87; 95% confidence intervals [CI], 1.84–4.46; \( p < 0.001 \)) for the mixed phenotype and 31.1% (HR, 4.92; 95% CI, 2.84–8.52; \( p < 0.001 \)) for the eosinophilic phenotype versus 80.9% for the clear phenotype (Fig. 2B). Mixed/eosinophilic phenotypes were significantly associated with worse pathological prognostic factors such as TNM stage, WHO/ISUP grade, necrosis, vascularity-based architectural category, and the immunophenotype (\( p < 0.001 \)) (Fig. 2C and Supplementary Table 2). The signature scores of angiogenesis gene, and ccA gene were significantly enriched in the clear phenotype compared with mixed/eosinophilic phenotypes (\( p < 0.001 \)) (Fig. 2D). In contrast, effector T-cell, immune checkpoint, and ccB gene signature scores were significantly enriched in mixed/eosinophilic phenotypes compared with the clear phenotype (\( p = 0.028, \ p < 0.001, \) and \( p = 0.011, \) respectively) (Fig. 2D). The myeloid gene signature was not significantly different between the phenotypes (\( p = 0.089 \)) (Fig. 2D).

Association of AI score based on transfer learning with clinicopathological characteristics and gene signatures in the development cohort

Representative clear or mixed/eosinophilic H&E images and corresponding class activation mappings of low or high AI scores are shown in Fig. 3A. The receiver operating characteristic (ROC) curve based on clear or mixed/eosinophilic phenotypes assessed by a pathologist showed the AI score cut-off was 0.579 (sensitivity 61.1%, specificity 72.5%) with an AUC of 0.733 (95% CI, 0.68–0.78) (Fig. 3B). Thus, an AI scores less than 0.579 were considered to have a low AI score, and an AI score greater than or equal to 0.579 was regarded as a high AI score. Low and high AI scores were seen in 175 (40.2%) and 260 (59.8%) patients, respectively. The high AI score group was associated with adverse pathological characteristics such as TNM stage, WHO/ISUP grade, necrosis, vascularity-based architectural category, and the immunophenotype (\( p < 0.01 \)) (Fig. 3C and Supplementary Table 3). The gene signature scores of angiogenesis gene, and ccA gene were significantly enriched in low AI scores compared with high AI scores (\( p = 0.005 \) and \( p < 0.001, \) respectively). In contrast, effector T-cell and immune checkpoint gene signature scores were significantly enriched in high AI scores compared with low AI scores (\( p = 0.011 \) and \( p < 0.001, \) respectively) (Fig. 3D). Myeloid and ccB gene signatures did not show a significant difference between the two scores (\( p = 0.478 \) and \( p = 0.073, \) respectively) (Fig. 3D).
Association of AI scores with histological features and gene signatures in the validation cohort

Representative H&E images of clear, mixed, and eosinophilic histological phenotypes and corresponding class activation mappings of AI scores are shown in Fig. 4A. The area under the ROC curve for predicting clear or mixed/eosinophilic phenotypes was 0.929 (95% CI, 0.88–0.98) (Fig. 4B). AI scores were significantly distributed into histological phenotypes, WHO/ISUP grade, and vascularity-based architectural categories assessed by a pathologist ($p<0.001$) (Fig. 4C). The signature scores of angiogenesis gene, and ccA gene were significantly enriched in the low AI score group compared with the high AI score group ($p<0.001$), whereas immune checkpoint, myeloid, and ccB gene signature scores were significantly enriched in the high AI score group compared with the low AI score group ($p = 0.026$, $p = 0.045$, $p < 0.001$, respectively). However, effector T-cell gene signatures were not significantly different between the two scores ($p = 0.539$) (Fig. 4D).

Prognostic significance of clear or mixed/eosinophilic phenotypes and the AI score in the TCGA-KIRC cohort

The Kaplan–Meier survival analysis showed that the five-year overall survival rate was significantly worse for patients with the mixed/eosinophilic phenotype than those with the clear phenotype (54.3% vs. 80.9%; $p < 0.001$) (Fig. 5A). Similarly, the five-year overall survival rate was significantly worse for patients with a high AI score than those with a low AI score (58.6% vs. 72.4%; $p = 0.008$) (Fig. 5B). The clear and mixed/eosinophilic phenotypes assessed by a pathologist showed a higher c-index for overall survival than the predicted AI score assessed by transfer learning (0.632 vs. 0.561) in the TCGA cohort.

Discussion

In this present study, we created a pipeline using transfer learning on a deep convolutional neural network to predict clear and eosinophilic (including mixed) phenotypes from the manually extracted highest-grade regions of whole-slide images. We revealed that the high AI score group, representing the predicted eosinophilic phenotype, significantly correlated with adverse pathological factors and gene signatures related to the responsiveness to checkpoint blockade. Our AI model detected clear and eosinophilic phenotypes with high prediction in the validation cohort (AUC = 0.929) and stratified patients’ outcomes in the TCGA-KIRC cohort ($p = 0.008$).

It can be challenging to define clear and eosinophilic phenotype on H&E-stained slides because the preparation of the pathological specimens and staining methods which often vary among institutions. In this current work, we successfully validated the clinical significance of clear or eosinophilic histological phenotypes, shown in our prior single-center study [4], via transfer learning using multi-institutional H&E images with color variations for clear and eosinophilic phenotypes from the TCGA-KIRC dataset. Furthermore, we confirmed that clear and eosinophilic phenotypes, predicted by our AI model, correlated with clinicopathological characteristics and gene signatures associated with angiogenesis and cancer
immunity and were in accordance with the results of the urologic pathologist’s manually assessed histological phenotypes. Our findings suggest that identifying clear or eosinophilic phenotypes may facilitate prognostic predictions and treatment selections.

To the best of our knowledge, this is the first histology-based AI model using H&E images to predict clear or eosinophilic phenotypes, correlated with gene signatures related to current treatment options and prognosis. Although the application of AI for diagnosis and prognostic predictions in renal cancers has been recently developed [9], previous AI algorithms were mainly focused on distinguishing the histological subtypes [10, 22], stages [23, 24], survival [25, 26], and nuclear grades [27, 28] of ccRCC. Recently an AI prediction model based on gene signatures has been developed using RNA sequencing data from the TCGA dataset and may be helpful in treatment and surveillance decisions [23, 29]. However, a molecular-based AI model is far from being implemented in routine clinical practices.

Recently, Chen et al. trained a deep convolutional neural network on H&E histology images available from the TCGA to classify EMT molecular classifications of ccRCC, characterized by distinctive genomes, metabolic states, and immune components [20]. Their approach distinguished the prediction of epithelial or mesenchymal subtypes in H&E slides and suggested that patients in the mesenchymal subtype might respond better to checkpoint blockades combined with angiogenetic therapies [20]. Similar to their study, our research group previously reported that mixed or eosinophilic phenotypes of ccRCCs showed upregulation of EMT gene signatures, a higher prevalence of sarcomatoid/rhabdoid features, and poor responsiveness to angiogenetic therapy [4].

While clear and eosinophilic phenotypes may be analogous to epithelial and mesenchymal subtypes in terms of underlying genomes and treatment response, histological features of epithelial and mesenchymal subtypes, corresponding to eosinophilic and clear cytoplasmic features, respectively, were the opposite in our results. Nevertheless, we believe that eosinophilic phenotypic areas tend to dedifferentiate, such as EMT, according to previous findings that eosinophilic phenotypic areas molecularly and immunohistochemically displayed a lower differentiation compared to clear phenotypic areas [4, 5]. We showed that ccRCC with eosinophilic phenotypes assessed by both a pathologist and the AI model significantly correlated with adverse pathological factors such as higher WHO/ISUP grades, vascularity-based architectural categories including sarcomatoid/rhabdoid components, and ccB gene signatures, consisting of genes related to regulating EMT.

We demonstrated that manually assessed histological phenotypes and the AI model predicted phenotypes were stratified with patients’ outcomes ($p < 0.0001$ and $p = 0.008$, respectively). However, the predictive ability of manually assessed phenotypes outperformed the ability of the AI model. We found some discrepancies between the assessments by the pathologist and our AI model (Supplementary Figs. 2A-2E). We found that small-sized clear neoplastic cells with pale reticular pink cytoplasm were sometimes identified as an eosinophilic phenotype, resulting in overestimating the clear phenotype. (Supplementary Fig. 2A) and the AI model included various components of the tumor microenvironment such as tumor vasculature, stromal, and tumor-associated immune cells into tumor components.
(Supplementary Fig. 2B). We also discovered that when the class activation mapping of AI scores corresponded to the H&E images, discordances between manually assessed histological phenotypes and AI scores were observed because of the defined cut-off value (Supplementary Figs. 2C-2E). Unlike the quantitative AI scores, clear, mixed, and eosinophilic phenotypes were assessed according to the WHO/ISUP grading and is determined based on the highest grade within a single high-power field of the tumor [15].

Our study had some limitations. First, we manually selected the highest-grade area for transfer learning according to the previously described method [4]. Second, we applied the AI model to the highest-grade area, not whole-slide images. Third, the cut-off of low or high AI scores was defined clear or mixed/eosinophilic phenotypes assessed by a pathologist. Especially, tumors with mixed phenotypes included various quantities of eosinophilic phenotypic areas tended to show discordances between manual and automatic methods. Fourth, the validation set did not include prognostic information because this study set was for confirming the correlation between histological features and gene signatures. Fifth, gene sets included in the custom gene panel examined in the present study were used in our previous studies [4, 14]. Lastly, we did not apply our AI model to treatment responsiveness. Despite these limitations, we showed meaningful correlations among our AI model, histological features, gene signatures, and patients’ outcomes. Although trained deep learning algorithms, referred to as black-box algorithms [30], cannot be easily explained, larger and more complex neural networks should be employed in future studies to solve the discordances between the pathologist’s assessment and the predictions from the AI model.

In conclusion, we successfully developed an AI model for predicting clear or eosinophilic histological phenotypes, which correlated with clinicopathological characteristics, gene signatures, and prognosis.

**Declarations**

**COMPETING INTERESTS:** CO received research funding from Chugai Pharmaceutical Co. Ltd. outside the submitted work. The remaining authors have no conflict of interest related to this work.

**ACKNOWLEDGEMENTS**

We are grateful to Mr. Ryosuke Yamaka for his technical assistance in the construction of tissue microarray, tissue sampling, and NanoString assay.

**ETHICS APPROVAL**

This study was performed in accordance with the Declaration of Helsinki. The ethics committee of Kansai Medical University approved this study (No. 2018109 and No. 2020222).

**AUTHOR CONTRIBUTION**
Conception and design: CO, TY, and HK. Acquisition, analysis, or interpretation of data: CO, TY, RU, NA, YY, JI, TN, YN, KH. Study supervision: MA, HK, KT. Manuscript writing: CO. Final approval of manuscript: All authors.

FUNDING

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DATA AVAILIBILITY

The data are available upon reasonable request by contacting the corresponding author.

CONFLICT OF INTERESTS

CO received research funding from Chugai Pharmaceutical Co. Ltd. outside the submitted work. The remaining authors have no conflict of interest related to this work.

References


**Figures**
Study workflow. As a development cohort, we used the TCGA-KIRC cohort, including whole-slide images, RNA-sequencing data, and survival information. First, histological evaluations were performed by a urologic pathologist. Second, the highest-grade area was extracted. Third, selected regions were cropped into tiles to develop the AI model. Fourth, the AI model was trained using transfer learning on a deep convolutional neural network, and clear or eosinophilic predictions were scaled as AI scores. Fifth, we verified the AI model using a validation cohort. Finally, we evaluated the prognostic predictions of the AI model.

H&E, hematoxylin and eosin; ccRCC, clear cell renal cell carcinoma; TMA, tissue microarray; AI artificial intelligence.
Figure 2

**A** Representative H&E-stained images of clear, mixed, and eosinophilic histological phenotypes.

**B** The Kaplan-Meier survival analysis of the TCGA cohort stratified by histological phenotypes.

**C** Percentage of cases of clear and mixed/eosinophilic phenotypes assessed by a pathologist and pathological characteristics.

**D** Comparison of gene signature scores (mean Z-score) between clear and mixed/eosinophilic phenotypes assessed by a pathologist. Mann-Whitney $U$ test was used for statistical analyses.

**Figure 2**

Association of clear and eosinophilic histological phenotypes assessed by a pathologist with clinicopathological characteristics and gene signatures in the development cohort ($n = 435$)

**A** Representative H&E-stained images of clear, mixed, and eosinophilic histological phenotypes. **B** The Kaplan-Meier survival analysis of the TCGA cohort stratified by histological phenotypes. **C** Percentage of cases of clear and mixed/eosinophilic phenotypes assessed by a pathologist and pathological characteristics. **D** Comparison of gene signature scores (mean Z-score) between clear and mixed/eosinophilic phenotypes assessed by a pathologist. Mann-Whitney $U$ test was used for statistical analyses.
Figure 3

**Association of AI scores based on transfer learning with clinicopathological characteristics and gene signatures in the development cohort (n = 435)**

**A** Representative H&E-stained images (a clear, c mixed/eosinophilic) and corresponding class activation mappings of low and high AI scores, visualized as cold (blue) or warm (red) colors, respectively. (b AI score: 0.0000, d AI score: 0.7137). **B** The ROC curve based on clear or mixed/eosinophilic assessed by a pathologist showing the cut-off value of the AI score. **C** Percentage of cases of low and high AI scores and pathological characteristics. **D** Comparison of the gene signature scores (mean Z-score) between low and high AI scores. Mann-Whitney *U* test was used for statistical analyses. AI artificial intelligence.
Figure 4

Association of AI scores based on transfer learning with histological features and gene expression in the validation cohort (n = 95)

A Representative H&E-stained images of TMA cores (top column) and corresponding class activation mappings of AI scores (bottom column), visualized as cold (blue) to warm (red) colors. (a) clear phenotype, AI score: 0.0551 (b) mixed phenotype, AI score: 0.3932, (c) eosinophilic phenotype, AI score: 0.9548 (d) eosinophilic phenotype, AI score: 0.9531. B The AUC for predicting clear or mixed/eosinophilic phenotypes was 0.929. C Percentage of cases of low and high AI scores and histological features. D Comparison of gene signature scores (mean Z-score) between low and high AI scores. Mann-Whitney U test was used for statistical analyses. AI artificial intelligence.
Figure 5

The Kaplan-Meier survival analysis of two approaches in the TCGA cohort (n = 435)

A, B The Kaplan-Meier survival analysis of clear and mixed/eosinophilic phenotypes (A) and low and high AI scores (B).

Supplementary Files

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- AIRCCSupplementaryinformationforsubmission.pdf