Original Research

Predicting oncogene mutations of lung cancer using deep learning and histopathologic features on whole-slide images

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A B S T R A C T

Lung cancer is a leading cause of death in both men and women globally. The recent development of tumor molecular profiling has opened opportunities for targeted therapies for lung adenocarcinoma (LUAD) patients. However, the lack of access to molecular profiling or cost and turnaround time associated with it could hinder oncologists’ willingness to order frequent molecular tests, limiting potential benefits from precision medicine. In this study, we developed a weakly supervised deep learning model for predicting somatic mutations of LUAD patients based on formalin-fixed paraffin-embedded (FFPE) whole-slide images (WSIs) using LUAD subtypes-related histological features and recent advances in computer vision. Our study was performed on a total of 747 hematoxylin and eosin (H&E) stained FFPE LUAD WSIs and the genetic mutation data of 232 patients who were treated at Dartmouth-Hitchcock Medical Center (DHMC). We developed our convolutional neural network-based models to analyze whole slides and predict five major genetic mutations, i.e., BRAF, EGFR, KRAS, STK11, and TP53. We additionally used 111 cases from the LUAD dataset of the CPTAC-3 study for external validation. Our model achieved an AUROC of 0.799 (95% CI: 0.686–0.904) and 0.686 (95% CI: 0.620–0.752) for predicting EGFR genetic mutations on the DHMC and CPTAC-3 test sets, respectively. Predicting TP53 genetic mutations also showed promising outcomes. Our results demonstrated that H&E stained FFPE LUAD whole slides could be utilized to predict oncogene mutations, such as EGFR, indicating that somatic mutations could present subtle morphological characteristics in histology slides, where deep learning-based feature extractors can learn such latent information.

Introduction

Lung cancer is a leading cause of death in both men and women in the world. In 2020, 1.8 million individuals have died from lung cancer, and 2.2 million cases are newly diagnosed [1]. Non-small cell lung carcinoma (NSCLC) accounts for more than 80% of lung cancer cases, and lung adenocarcinoma (LUAD) is one of the most prevalent histologic subtypes of NSCLC. The recent development of molecular profiling has opened new targeted therapy opportunities for LUAD patients, which can improve clinical outcomes and the quality of life of patients. Several actionable mutations have been identified for LUAD targeted treatment: KRAS, EGFR, ALK, MET, BRAF, RET, ROS1, NTRK, and ERBB2 [2,3]. The mutation frequencies and the clinical implications of each mutation in NSCLC patients vary. For example, EGFR somatic mutation is present in 12–15% of the Caucasian population with NSCLC and 47–64% of East Asian NSCLC patients. Its reported Overall Response Rate (ORR) to Osimertinib, a third-generation EGFR tyrosine kinase inhibitor (TKI), is about 80%. In contrast, the reported ORR of KRAS p.G12C mutation targeted drug, Sotorasib, is 32% [3].

Treatments targeting these mutations have improved the survival rate of NSCLC male patients from 26% in 2001 to 35% in 2014 [4]. Next-generation sequencing (NGS) testing, which is performed on tumor tissue samples to identify somatic mutations, is used in the current standard of care for advanced NSCLC patients [5]. In a recent survey in the United States, over 75% of oncologists use NGS tests to guide their treatment decisions for patients [6]. This survey, however, also revealed the low frequency of ordering NGS testing. While the cause of the low frequency of NGS testing is not well studied, lack of access, long turn-around time (typically 10–14 days), and the cost of testing could hinder the oncologists’ willingness to order NGS testing. There are potentially more patients who could benefit from performing tumor molecular profiling to decide treatment eligibilities in precision oncology.

There has been a new interdisciplinary development at the intersection of clinical oncology and machine learning research to predict the actionable mutations in cancer tissues based on formalin-fixed paraffin-embedded (FFPE) whole-slide images without the need for molecular
profiling tests for colorectal cancer [7], gastric cancer [8], breast cancer [9,10] and lung cancer [11–13]. Coudray et al. applied a convolutional neural network (CNN) to predict ten major mutations and demonstrated that predicting the mutation status of $STK11$, $EGFR$, $FAT1$, $SETBP1$, $KRAS$, and $TP53$ is a feasible task [11]. In recent work, Chen et al. presented a two-stage CNN model to predict $EGFR$ and KRAS mutations and achieved an AUC of 0.683 and 0.545 for $EGFR$ and KRAS on their test set, respectively [14]. Another study by Huang et al. achieved an AUC of over 0.750 for predicting $FGFR1$, $FGFR2$, HRAS, and MET mutations on a TCGA-based test set using a CNN [15]. In this study, we set up a novel weakly supervised framework to train a deep learning model for patient-level somatic mutation prediction with validation on both internal (DHMC) and external (CPTAC-3) datasets, adding new methodological improvements and experimental evidence to the existing body of work to advance this field. We hypothesize that LUAD subtype-related histopathology features extracted using a CNN could be utilized to further predict the oncogene mutations. To this end, we develop a new weakly supervised deep learning model for predicting somatic mutations based on FFPE whole-slide images (WSIs) of LUAD patients. Ultimately, the successful development of such algorithms to identify oncogene mutations based on whole slide images would be a great benefit for both patients and healthcare systems by providing a triaging method that could be utilized before performing time-intensive and expensive molecular testing to screen patients for clinically actionable mutations and identify and prioritize those cases that likely benefit from targeted treatments in a more timely manner.

Methods

Datasets

A total of 747 hematoxylin and eosin (H&E) stained FFPE lung adenocarcinoma (LUAD) whole-slide images and their corresponding genomic profile were collected from 232 patients who were treated at the Dartmouth Hitchcock Medical Center between 2018 and 2019. We included all the H&E stained FFPE tumor slides available for each patient in our dataset for this study. Of note, most patients had less than five slides in our dataset, while a few patients had more than ten slides. These H&E-stained slides were digitized by an Aperio AT2 scanner (Leica Biosystems, Wetzlar, Germany) at 40x magnification (0.25 μm/pixel). The genetic mutation data was generated by next generation sequencing (NGS) as part of routine patient treatment at DHMC. The NGS panel used for these samples covered hotspot mutation regions in 50 cancer related genes. These 50-gene hotspot regions cover the most relevant genetic information for precision-medicine lung-cancer management. These regions and their relevance to lung cancer were established through a rigorous independent research and selection process, which included a systematic review (by multiple domain expert genetic, pathology, and oncology researchers at DHMC and Norris Cotton Cancer Center) of the most prominent NSCLC knowledge bases, such as the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology, My Cancer Genome: Genetically Informed Cancer Medicine, COSMIC: Catalogue of Somatic Mutations in Cancer, ClinVar National Center for Biotechnology Information, dbSNP National Center for Biotechnology Information, and literature searches using PubMed [16–23]. Curation of these hotspot regions and determination of their clinical importance are previously published and established [16–22].

We binarized the NGS data for these 50-gene hotspots to indicate whether a mutation for each gene was present. We considered five genes, $BRAF$, $EGFR$, $KRAS$, $STK11$, and $TP53$. In this study because those were the genes that were mutated in at least five percent of all patients in our dataset. Three oncogenes, $BRAF$, $EGFR$ and $KRAS$, are typically mutually exclusive. However, we identified two cases in our dataset with mutations in both $BRAF$ and $KRAS$. This overlap has also been reported in previous studies on these mutations in NSCLC patients [24]. Of note, two tumor suppressor genes, $TP53$ and $STK11$, can overlap with the oncogenes, particularly $TP53$. In our dataset, we found 24 co-occurrences of mutations in $KRAS$ and $TP53$, 7 co-occurrences of mutations in $BRAF$ and $TP53$, 6 co-occurrences of mutations in $KRAS$ and $STK11$, 4 co-occurrences of mutations in $KRAS$, $STK11$, and $TP53$, 2 co-occurrences of mutations in $BRAF$, $KRAS$, and $TP53$, and 1 co-occurrence of mutations in $EGFR$ and $STK11$. We randomly partitioned the slides stratified by patient into train, validation, and test set, containing 471, 97, and 179 cases, respectively. Due to the heterogeneous distribution of genetic mutations in our dataset, we ensured each partition included at least one patient with mutation for these five genes. Table 1 summarizes the distribution of patients and their genetic mutation status in our dataset.

Additionally, we collected 140 H&E stained FFPE slides and corresponding genetic mutation data of 111 lung cancer patients from the Clinical Proteomic Tumor Analysis Consortium 3 (CPTAC-3), as external validation [25]. CPTAC-3 dataset has up to two tumor slides for each patient, which are all included in our analysis. This study and the use of human participant data in this project were approved by the Dartmouth-Hitchcock Health Institutional Review Board (IRB) with a waiver of informed consent. The conducted research reported in this study is in accordance with this approved Dartmouth-Hitchcock Health IRB protocol and the World Medical Association Declaration of Helsinki on Ethical Principles for Medical Research involving Human Subjects.

Data preprocessing

Since digitized slides consist of multi-million pixels, which current common computational hardware cannot easily process at once, we preprocessed each whole-slide image in our dataset and extracted smaller fixed-size patches for our analysis. For this preprocessing, we first down-sampled each whole-slide image by a factor of eight (i.e., converted the images to 5x magnification or 2.0 μm/pixel), removed background and artifacts, and generated patches of 224×224 pixels using a sliding window approach [26] with an overlapping factor of 1/3 from these down-sampled whole-slide images.

We applied patch filtering with a CNN model pre-trained on a LUAD dataset for histologic subtype classification task [26] to remove an overwhelming number of patches with normal tissue and focused on LUAD-related regions of the whole slides. For this filtering, we applied the aforementioned pre-trained model [26] to predict the histological subtype of the patch (i.e., acinar, lepidic, micropapillary, papillary, solid, or normal), and removed patches predicted as normal. The patch filtering method removes normal patches that have less to no information about the tumor and effectively accelerates the model training by reducing the number of training samples. In addition, since we used a weakly supervised framework for CNN model training, removing normal patches was an essential preprocessing step for noise reduction.

Deep neural network based models

We took a bottom-up approach to analyze whole-slide images, where a set of fixed-size (i.e., 224 × 224 pixels) tissue patches from a digitized

| Table 1 | The distribution of patients and their mutation status in our datasets. |
|---------|-----------------------------------------------------------------------|
|         | DHMC Training Set | Validation Set | CPTAC-3 Test Set 1 | Total | Test Set 2 |
| No. of Patients | 148 | 24 | 60 | 232 | 111 |
| Mutations | | | | | |
| $KRAS$ | 63 | 14 | 21 | 98 | 34 |
| $TP53$ | 60 | 5 | 25 | 90 | 59 |
| $STK11$ | 12 | 3 | 4 | 19 | 20 |
| $BRAF$ | 9 | 1 | 3 | 13 | 6 |
| $EGFR$ | 17 | 1 | 9 | 27 | 37 |
| No. of Slides | 471 | 97 | 179 | 747 | 140 |
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of 0.69 (95% CI: 0.578–0.777) for TP53 mutation on the internal DHMC test set. The models, however, did not perform better than an AUC of 0.6 on the external CPTAC-3 test set.

**Discussion**

In this study, we developed several deep neural network-based models that predict oncogene mutations based on H&E stained FFPE whole-slide images of LUAD patients to investigate the utilization of existing pretrained models that were developed for a different task. Our experiments showed promising performance in predicting EGFR and TP53 mutations, achieving an AUC of 0.799 and 0.713 on the internal DHMC test set, using an ImageNet pretrained CNN model with fine-tuning all the layers. A model that fine-tuned only the last fully-connected layer and directly reused the generic image-based features did not achieve the best performance, indicating that ImageNet-based generic image features might not be directly applicable to our task. A model with ImageNet parameters, however, could be a good starting point to fine-tune for further domain-specific tasks, confirming a common ground in deep learning research \[28,29\]. We also observed that a model using LUAD features had limited generalizability in predicting BRAF and TP53 mutations when tested on the external test set.

**Table 2**

| Models                     | BRAF   | EGFR   | KRAS   | STK11  | TP53   |
|----------------------------|--------|--------|--------|--------|--------|
| CNNLUAD-Feat-FT/FC         | 0.804  | 0.627  | 0.604  | 0.612  | 0.711  |
| (0.614–0.972)              | (0.483–0.764) | (0.490–0.719) | (0.407–0.800) | (0.616–0.803) |
| CNNLUAD-Feat-FT/AL         | 0.483  | 0.570  | 0.530  | 0.606  | 0.635  |
| (0.197–0.767)              | (0.446–0.687) | (0.419–0.639) | (0.392–0.802) | (0.528–0.741) |
| CNNImage-Feat-FT/FC        | 0.518  | 0.479  | 0.474  | 0.521  | 0.565  |
| (0.244–0.780)              | (0.348–0.605) | (0.364–0.582) | (0.262–0.769) | (0.456–0.672) |
| CNNImage-Feat-FT/AL        | 0.543  | 0.799  | 0.596  | 0.609  | 0.713  |
| (0.297–0.774)              | (0.686–0.904) | (0.486–0.706) | (0.374–0.832) | (0.611–0.811) |

**Table 3**

| Models                     | BRAF   | EGFR   | KRAS   | STK11  | TP53   |
|----------------------------|--------|--------|--------|--------|--------|
| CNNLUAD-Feat-FT/FC         | 0.456  | 0.605  | 0.598  | 0.527  | 0.475  |
| (0.270–0.638)              | (0.530–0.680) | (0.519–0.677) | (0.428–0.625) | (0.392–0.558) |
| CNNLUAD-Feat-FT/AL         | 0.423  | 0.500  | 0.601  | 0.438  | 0.570  |
| (0.241–0.601)              | (0.425–0.576) | (0.518–0.682) | (0.334–0.540) | (0.486–0.654) |
| CNNImage-Feat-FT/FC        | 0.550  | 0.513  | 0.457  | 0.478  | 0.400  |
| (0.411–0.690)              | (0.462–0.563) | (0.378–0.535) | (0.373–0.581) | (0.324–0.476) |
| CNNImage-Feat-FT/AL        | 0.451  | 0.686  | 0.629  | 0.484  | 0.677  |
| (0.270–0.628)              | (0.620–0.752) | (0.552–0.706) | (0.406–0.562) | (0.602–0.752) |

Fig. 2. The receiver operating characteristic curves (ROC) of CNNImage-Feat-FT/AL for each somatic mutation predicted for (a) internal DHMC test set and (b) external CPTAC-3 test set. Each column from left to right corresponds to BRAF, EGFR, KRAS, STK11, and TP53, respectively. The gray bands represented 95% confidence intervals of each ROC.
LUAD dataset in this study were collected independently, we hypothesize that there might exist some internal consistency, such as tissue preparation or scanner type, that could lead to models’ overfitting. Also, it is worth noting that we did not observe any statistically significant difference in performance for patients with and without co-occurring mutations based on our results.

The prediction performance of our models is not directly comparable with those of previous work due to the use of different datasets; however, our promising performance in predicting EGFR mutations is aligned with the previous work [11,14]. Of note, EGFR mutations, which one of our proposed models identifies at an AUC of 0.799 based on whole-slide images in the DHMC test set, is an important factor in the targeted treatment of NSCLC patients. Currently, Osimertinib, an EGFR inhibitor, is approved by the US Food and Drug Administration (FDA) for the treatment of NSCLC with stage IB and above and have led to improvements in clinical outcome and quality of life of NSCLC patients with EGFR-mutation [30,31].

Any delay in genetic testing of NSCLC patients with potential clinically-actionable mutations, such as EGFR, can have major impacts on patients clinical outcomes. We expect further development and validation of the presented methods in this work could lead to new approaches to identify NSCLC patients with clinically-actionable mutations based on tumor pathology slides, and to provide an accurate, fast, and inexpensive pre-selection method that could be utilized before performing time-intensive and expensive genetic tests to screen patients for clinically-actionable mutations. As a result, these prediction methods could prioritize genetic screening of NSCLC patients who are the most likely to have clinically-actionable mutations, thus reducing screening turnaround time and increasing the accuracy of treatment administration. In addition, such pre-selection methods could improve the finding and tracking of NSCLC patients with clinically-actionable mutations for translational research, as well as facilitate the recruitment of NSCLC patients for clinical trials.

Our study further supports oncogene mutation prediction using deep learning with both internal and external test sets, suggesting that gene mutations could present subtle morphological characteristics in whole slides, where deep learning-based feature learners can extract such latent information. Of note, utilizing histopathology features of LUAD subtypes had limited utility in predicting oncogene mutations. Still, our experiments showed promising results for predicting BRAF, EGFR, and TP53 mutations based whole-slide image features. As a future direction, we plan to investigate KRAS and STK11 mutations with alternative approaches. In addition, we plan on collecting larger multi-institutional LUAD datasets to investigate the predictability of specific mutations for each gene. Finally, we plan to extend our histopathology-based analysis to further predict response levels and time to the development of resistance for targeted therapies.

### Table 4

| Datasets | Models       | BRAF       | EGFR       | KRAS       | STK11      | TP53       |
|----------|--------------|------------|------------|------------|------------|------------|
|          | LogitLUAD-his-max | 0.456     | 0.66       | 0.506      | 0.5        | 0.661      |
| DHMC     |              | (0.295–0.592) | (0.540–0.754) | (0.353–0.633) | (0.346–0.628) | (0.541–0.754) |
|          | LogitLUAD-his-mean | 0.578     | 0.681      | 0.515      | 0.5        | 0.692      |
|          |              | (0.439–0.690) | (0.567–0.770) | (0.364–0.640) | (0.346–0.628) | (0.580–0.778) |
|          | LogitLUAD-his-min | 0.725     | 0.631      | 0.5        | 0.5        | 0.69       |
|          |              | (0.623–0.803) | (0.504–0.731) | (0.346–0.628) | (0.346–0.628) | (0.578–0.777) |
|          | LogitLUAD-his-max | 0.158     | 0.468      | 0.553      | 0.5        | 0.576      |
|          |              | (0.0–0.335) | (0.309–0.602) | (0.409–0.670) | (0.346–0.628) | (0.436–0.688) |
| CPTAC-3  | LogitLUAD-his-mean | 0.163     | 0.475      | 0.562      | 0.5        | 0.578      |
|          |              | (0.0–0.339) | (0.317–0.608) | (0.420–0.677) | (0.346–0.628) | (0.439–0.690) |
|          | LogitLUAD-his-min | 0.186     | 0.488      | 0.57       | 0.5        | 0.583      |
|          |              | (0.0–0.360) | (0.332–0.618) | (0.429–0.684) | (0.346–0.628) | (0.445–0.694) |

### Contributors

Concept and design: L.T. and S.H.; Acquisition, analysis, or interpretation of data: All authors; Drafting of the manuscript: M.N., N.T., and S.H.; Critical revision of the manuscript for important intellectual content: All authors; Statistical analysis: M.N. and N.T.; Obtained funding: S.H.; Administrative, technical, or material support: S.H.; Supervision: S.H.

### Data sharing statement

CPTAC-3 data can be downloaded from the website: https://portal.gdc.cancer.gov/projects/CPTAC-3. The DHMC dataset used in this study is not publicly available due to patient privacy constraints. An anonymized version of this dataset can be generated and shared upon reasonable request from the corresponding author.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Supplementary materials

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