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Stratification of resectable lung adenocarcinoma by molecular and pathological risk estimators

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Abstract ***Background:*** Mortality in early stage, resectable lung cancer is sufficiently high to warrant consideration of post-surgical treatment. Novel markers to stratify resectable lung cancer patients may help with the selection of treatment to improve outcome.
Methods: Primary tumour tissue from 485 patients, surgically treated for stage I–II lung adenocarcinoma, was analysed for the RNA expression of 31 cell cycle progression (CCP) genes by quantitative polymerase chain reaction (PCR). The expression average, the CCP score, was combined with pathological stage into a prognostic score (PS). Cox proportional hazards regression assessed prediction of 5-year lung cancer mortality above clinical variables. The PS threshold was tested for risk discrimination by the Mantel–Cox log-rank test.
Results: The CCP score added significant information above clinical markers (all patients, $P = 0.0029$; stage I patients, $P = 0.013$). The prognostic score was a superior predictor of outcome compared to pathological stage alone (PS, $P = 0.00084$; stage, $P = 0.24$). Five-year lung cancer mortality was significantly different between the low-risk (90%, 95% confidence interval (CI) 81–95%), and high-risk groups (65%, 95% CI 57–72%), $P = 4.2 \times 10^{-6}$).
Conclusions: The CCP score is an independent prognostic marker in early stage lung adenocarcinoma. The prognostic score provides superior risk estimates than stage alone. The

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threefold higher risk in the high-risk group defines a subset of patients that should consider therapeutic choices to improve outcome.

1. Introduction

In 2008, 13% of the cancer burden worldwide was attributable to lung cancer, causing 18% of all cancer related deaths [1]. With an estimated 224,000 new cases in 2014, lung cancer is the second most common cancer for both males and females and the leading cause of cancer deaths in the US, and despite novel treatment options survival rates for lung cancer remain poor [2] With further population ageing, a 67% increase in the incidence of lung cancer has been predicted for patients aged $\geqslant$65 years by 2030 [3]. Improved imaging sensitivity and scanning of high-risk groups has facilitated detection of lung cancers at an earlier stage where curative surgery remains an option [4,5]. In addition, the introduction of less invasive surgical methods has been shown to reduce complications and co-morbidities and to enhance the feasibility of adjuvant treatment [6,7].

For patients diagnosed with localised non-small cell lung cancer (NSCLC), surgical excision remains the cornerstone of treatment. Recurrences, however, are frequent for all stages and as many as 40% of stage I and 66% of stage II patients will die within 5 years from surgery [8]. Trials examining the efficacy of adjuvant chemotherapy in early stage NSCLC indicated that the benefit of adjuvant chemotherapy is related to tumour stage [9–11], and current treatment guidelines recommend adjuvant chemotherapy for stage II and IIIA patients [12,13]. However, a significant proportion of stage I NSCLC recur after surgery alone and only a fraction of stage II patients can be helped by cytotoxic therapy. Gene profiling studies of different cancer types have demonstrated that tumour biology plays a role in tumour behaviour and outcome. Assessment of the molecular portrait of cancer is likely to add prognostic discrimination to the time-dependent staging information.

Adenocarcinoma is the most common histologic subtype of NSCLC in the US and many molecular RNA expression profiles assert prognostic discrimination in the adenocarcinoma subtype [14–19]. Such an expression-based prognostic would be clinically useful if it is more effective than clinical variables in identifying either stage IA patients at high-risk who might need adjuvant chemotherapy or stage IB/II patients who have low risk of recurrence in the absence of adjuvant therapy. However, few expression signatures in lung cancer have shown consistent results, convincing evidence of superiority to clinical variables or applicability in formalin-fixed clinical samples and none has become part of routine clinical practice [18,20].

In previous studies we have described an RNA expression signature of proliferation genes (the cell cycle progression score or cell cycle progression (CCP) score) as an independent prognostic marker in lung adenocarcinoma and other cancers [20–22]. A subsequent study detailed the creation of a prognostic model combining the CCP score and pathological stage into the prognostic score (PS) [22]. The prognostic score was validated as a superior risk estimator compared to pathological stage alone in an independent data set [22]. In the study presented here, we use a third independent patient cohort to validate a risk threshold, designed to identify patients at low- or high-risk of lung cancer mortality based on their prognostic score.

2. Materials and methods

For this retrospective analysis patients were selected from observational cohorts by prospectively designed criteria and fixed tissues were analysed by a standardised protocol for an established set of expression markers. Molecular data were generated blinded to clinical and outcome data. Primary and exploratory goals of the study were pre-specified in a statistical analysis plan.

2.1. Patients and samples

FFPE tissue blocks from primary tumours of patients treated with surgical resection with curative intent were retrieved from three sources: the Nottingham Health Science Biobank (NHU), the Hospital-Integrated Biobank (BB-0033-00025) at the Centre Hospitalier Universitaire de Nice (CHUN) and the registered lung cancer sample collection (C0000960) at the Center for Applied Medical Research from the University of Navarra Hospital-Clínica Universidad de Navarra (CIMA-CUN). All patients were selected based on clinical records as having been diagnosed with primary non-metastatic adenocarcinoma of the lung with pathological tumour stages I and II (T1a-T3N0 and T1a-T2bN1) according to the 7th edition TNM criteria [8]. Patients had been consented for use of tissue specimen in biomedical research under protocols established at each institution. Samples were excluded from this analysis for any of the following reasons: stage III or IV disease, neo-adjuvant chemotherapy, non-adenocarcinoma histology, incomplete resection, multiple primary lung tumours, previous or synchronous lung tumours, missing clinical data or missing clinical follow-up. Patients who were known to have received adjuvant chemotherapy