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ANATOMICAL PATHOLOGY

Low tumour cell content in a lung tumour bank: implications for molecular characterisation

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Summary

Lung cancer encompasses multiple malignant epithelial tumour types, each with specific targetable, potentially actionable mutations, such that precision management mandates accurate tumour typing. Molecular characterisation studies require high tumour cell content and low necrosis content, yet lung cancers are frequently a heterogeneous mixture of tumour and stromal cells. We hypothesised that there may be systematic differences in tumour cell content according to histological subtype, and that this may have implications for tumour banks as a resource for comprehensive molecular characterisation studies in lung cancer. To investigate this, we estimated tumour cell and necrosis content of 4267 samples resected from 752 primary lung tumour specimens contributed to a lung tissue bank. We found that banked lung cancer samples had low tumour cell content (33%) generally, although it was higher in carcinoids (77.5%) than other lung cancer subtypes. Tumour cells comprise a variable and often small component of banked resected tumour samples, and are accompanied by stromal reaction, inflammation, fibrosis, and normal structures. This has implications for the adequacy of unselected tumour bank samples for diagnostic and molecular investigations, and further research is needed to determine whether tumour cell content has a significant impact on analytical results in studies using tissue from tumour bank resources.

Key words: Lung cancer; tumour; heterogeneity; tissue bank; pathology.

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INTRODUCTION

Lung cancer encompasses several malignant epithelial tumour types, as defined in the World Health Organization (WHO) Classification of Tumours,[1] the most common of which are adenocarcinomas (AdC) and squamous cell carcinomas (SCC). Optimal management mandates accurate histological subtyping and molecular characterisation to identify actionable mutations within each subtype.[2] Therefore, pathologists face the challenge of allocating small volume biopsy samples to those tests which maximise patient outcomes.[3] With anticipated innovations in personalised medicine, the amount of tissue required for patient-specific assays will likely increase even further.

Carcinomas are a variable and heterogeneous mixture of tumour and host cells,[4] which in small biopsies with low tumour cell content, can make tumour diagnosis difficult.[5] In addition, the number of viable cells available for examination is compromised in tissue samples with a high proportion of necrosis. Gellert *et al.*[6] found that taking five or more bronchoscopic biopsy samples from centrally-located tumours was associated with 90% probability of at least one biopsy containing tumour. However, often one or more bronchoscopic biopsies from endobronchial or distal lung tumours contain no tumour,[7] instead comprising tumour stroma or non-malignant bronchial mucosa. This highlights the dilemma that small biopsies adequate for broad histological diagnosis may be insufficient for subtyping and/or molecular testing.[8]

Banking tissue from surgical specimens[9] and bronchoscopic samples[10] allows for future research to develop new diagnostic and therapeutic strategies. However, if the tumour cell content of stored samples is unknown, it is uncertain whether they are adequate for these purposes. Techniques such as digitally guided or laser capture microdissection to isolate subpopulations of cells can increase the chance of detecting genetic mutations[11,12] or specific protein patterns[13] that may only be displayed in certain parts of a tumour. Large scale genomic projects use specific criteria for tumour sample eligibility. Therefore, results from such projects need to be interpreted in the knowledge that having been selected for tumours with high tumour cell content and low necrosis content, they may not be representative of the whole spectrum of lung cancers. To investigate the validity of this concern we quantified tumour cell and necrosis content in resected primary lung cancer tissue samples that were contributed to a tumour bank. The aims were to determine the general suitability of tumour bank specimens for molecular characterisation studies, and to determine whether there were systematic differences in tumour cell content and necrosis content between major lung cancer histological subgroups.

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MATERIALS AND METHODS

TPCH Lung Bank

The Prince Charles Hospital (TPCH) is a 630 bed hospital and a major Australian centre for thoracic surgery, undertaking >200 lung resections per year over the past 10 years. Since 1990, the University of Queensland Thoracic Research Centre (UQ-TRC) has invited patients undergoing resections at TPCH for primary lung cancer and a variety of other conditions to donate resected tissue not required for diagnostic purposes to TPCH Lung Bank for research via written informed consent. These donations were approved by TPCH Human Research Ethics Committee. Here we describe the contents of the repository collected between 1991 and 2014. During this time, 1378 specimens overall were accrued in TPCH Lung Bank; an average of ~65 specimens per year. Of these, 752 primary lung cancers with sufficient tissue and accompanying identifying data were selected for analysis in this study.

Specimen collection, processing, and sectioning

Primary lung tumour specimens were excised from resected tissue by an experienced anatomical pathologist with minimal delay post-surgery (Supplementary Fig. 1, Appendix A). Samples of each specimen not required for diagnosis were selected for storage in TPCH Lung Bank. If patients consented to donate resected tumour tissue but the entirety of the tumour was required for diagnostic purposes, no samples were banked. Tissue was harvested from regions macroscopically anticipated to contain tumour with minimal necrosis, and was divided into one or more samples of approximately 1–20 mm in length and snap-frozen in liquid nitrogen at −80°C, reflecting the method for fresh-frozen specimen preservation proposed by The Cancer Genome Atlas (TCGA; http://cancergenome.nih.gov/). In general, larger specimens were divided into more samples compared to smaller specimens, without adherence to a minimum number. A 2 mm slice of tissue was harvested from one margin of each banked tumour sample, placed into an embedding cassette and fixed in 10% neutral buffered formalin (Australian Biostain, Australia) for 3 h at 45°C (Supplementary Fig. 1, Appendix A). This was processed overnight in either a Peloris (Leica Biosystems, Germany) or Sakura Tissue-Tek VIP 6 (Sakura, The Netherlands) tissue processor according to the manufacturer's instructions, using ethanol as a dehydrator and xylene for clearing. Each sample portion was embedded in an individual block of paraffin wax using a Leica EG1160 embedding station (Leica Biosystems).

Tumour cell content and necrosis assessment

To obtain stained sections for estimation of tumour cell and necrosis content, 4 µm sections were cut from each block with either a Leica RM2145 or RM2245 microtome (Leica Biosystems), mounted on glass slides, heat fixed for 8 min, and then processed for standard haematoxylin and eosin (H&E) staining. One H&E stained section from each tissue block was independently evaluated by each of two pathologists for tumour cell and necrosis content (Supplementary Fig. 1, Appendix A). Estimated tumour cell content (TC) was scored as the percentage of viable cells that were tumour cells. Necrosis content (NC) was recorded as the percentage area of the whole tissue section that was necrotic. One pathologist used a millimetre grid to measure each section to aid in estimating proportions and calculating surface areas, but no other technological enhancements were used. In addition, in tumours that were estimated as having <30% TC, one pathologist documented the types of cells and structures comprising the tumour environment (e.g., inflammation, fibrosis, blood vessels and necrosis).

Histological subtyping of lung cancers

The subtype of each tumour was determined from the original diagnostic pathology reports, key elements of which included immunohistochemistry staining for validated subtype-specific markers and morphological features according to the WHO Classification of Tumours.[1] Briefly, pathologists used the following criteria to determine subtypes:

- Key features of SCC include keratinisation and/or intercellular bridges as well as squamous differentiation.
- AdC tumours may be mucinous or show glandular differentiation in a variety of architectures, including bronchioloalveolar/lepidic, papillary, acinar or solid mucinous growth patterns, with the majority of AdC demonstrating a mix of subtypes.
- Adenosquamous carcinoma is a combined AdC and SCC tumour with a minimum of 10% of each component.
- Large cell carcinoma lacks the distinguishing features of AdC and SCC.
- Large cell neuroendocrine carcinoma is similarly poorly differentiated, but shows neuroendocrine differentiation confirmed by immunohistochemical markers such as chromogranin, synaptophysin and CD56.
- Small cell lung carcinoma (SCLC) is distinct from the other subtypes, being high-grade with neuroendocrine features and with smaller sized cells.
- A tumour generically designated a 'non-small cell carcinoma' (NSCLC), a term typically used in describing small biopsy specimens rather than resections, is one with no distinguishing features supporting subtyping, although the term 'NSCLC' is also sometimes used as an umbrella term referring to all carcinomas that are not SCLC.
- Carcinoid tumours are low grade malignancies of the lung showing neuroendocrine differentiation. They are divided based on the presence of mitotic activity and necrosis into typical and atypical carcinoids.

Statistical analysis

Average tumour cell and necrosis content data between the two pathologists was analysed using non-parametric median comparisons or bivariate Spearman two-tailed correlations on SPSS v24 software (SPSS, USA). One way intraclass correlation coefficient consistency calculations were used for reliability testing. A two-tailed p value of <0.05 was considered statistically significant.

RESULTS

Subtype histology distribution of primary lung cancers in TPCH Lung Bank reflect lung cancer histology subtype prevalence

A total of 4267 sample blocks from 752 primary lung cancer specimens stored in TPCH Lung Bank were assessed for tumour and necrosis content. The resected tumour specimens were derived from 411 AdC, 241 SCC, 24 carcinoids (7 atypical and 17 typical) and 76 other cancers (including 24 large cell carcinoma, 21 large cell neuroendocrine carcinoma and 31 adenosquamous carcinoma). The histology subtype distributions were AdC (55%), SCC (32%) and other (13%) of the total (Fig. 1A). The specimens ranged in size from 3 to 165 mm in diameter, with a mean diameter of 40.2 mm. The number of samples (and corresponding paraffin blocks) per specimen ranged from 1 to 85, with a median of 4 per specimen. There was a weak correlation between number of samples (and corresponding paraffin blocks) banked per specimen and the size of the resected tumour ($r = 0.256$, $p < 0.001$). The area of the tissue sections taken from each block ranged from 1 to 128 mm^2, with a median area of 15 mm^2 (Fig. 1B).

Agreement between the two pathologists for tumour cell and necrosis content scores was high

The independent scores assigned by the two pathologists were highly correlated for both tumour cell content (TC) and necrosis content [NC; intraclass correlation coefficient 0.926 ($p < 0.001$) and 0.960 ($p < 0.001$), respectively]. One pathologist assigned systematically higher TC scores than the other, while NC scores were similar between pathologists.

Most lung cancer subtypes have relatively low tumour cell content and little necrosis

The majority of lung cancer blocks had tumour cell content below 50% (3188/4267, 74.7%), but ranged from 0 to 100% in all subtypes. In tumour blocks scoring lower TC percentages than the median, 54.3% (2319/4267 total) had ≤30% TC, and 20.6% (877/4267 total) had ≤5% TC. Relatively low TC was observed in all lung cancer subtypes

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