Stratifying cancer treatment responses in mesothelioma with AI-driven bioimaging

Abstract

Cancers, as a result of distinct mutations, lead to the expansion of clonal cell populations that exhibit considerable heterogeneity in epigenetic, physical and transcriptome profiles. This pathogenic capacity enables their survival under nutrient-poor conditions, infiltration and metastasis and poses significant treatment challenges, particularly due to the emergence of therapy-resistant cell subpopulations. Therefore, an ability to analyse and predict cellular responses to therapeutics early, and in rare cell populations would be transformative. Our project aims to identify precisely why, and how cancer cells differentiate from healthy cells by integrating Artificial Intelligence (AI) with cutting-edge bioimaging modalities. Specifically, we will focus on pleural mesothelioma, a deadly cancer caused by asbestos exposure, characterised by mutations in key tumour suppressors [1, 2]. Using state-of-the-art label-free imaging platforms (Nanolive and Lifecyte), alongside confocal high content imaging (Opera Phenix Plus), we will perform high-resolution, multi-parametric analysis to understand the unique cellular dynamics and predict patient responses. This approach will leverage our recently developed isogenic cellular model that closely represents the disease, facilitating the development of personalised treatment strategies. Through continuous refinement and validation of our AI-driven model against a panel of promising drug candidates, this project aims to provide ground-breaking improvements in diagnosing and treating mesothelioma, potentially extending to other cancers with similar genetic disruptions. We anticipate that this project will enhance opportunities for stratification and early diagnosis, and contribute to the development of future therapeutic strategies.

Introduction

Pleural mesothelioma is a deadly cancer with no cure and is caused by loss of function mutations in tumour suppressors, usually in either *NF2*, or *BAP1*. Pleural mesothelioma is predominantly caused by the fibrous mineral asbestos. The disease manifests itself after a long latency period of around 30 years. The incidence rate of mesothelioma is still increasing, due to the widespread presence of asbestos in buildings, including schools, combined with the continued mining and use of asbestos in some populous countries. Noteworthy, some nanofibers in rodent models, also appear to cause mesothelioma-like disease. Scotland has a grave history of pleural mesothelioma. Glasgow was once named the asbestos capital of Europe, due to asbestos used in the shipbuilding communities along the Clyde. Scotland therefore has one of the highest rates of pleural mesothelioma in Europe.

Designing therapeutics against the loss of tumour suppressors is challenging [4]. Consequently, there is a continued urgent need to innovate in order to identify curative therapeutics. In this pursuit, the Hansen lab seeks to identify new therapeutic targets and therapeutic approaches and has ongoing projects to obtain better treatments for mesothelioma patients, including seeking to stratify these and develop targeted therapies [2, 4].

Cancer and healthy cells respond differently depending on the contextual input received within the cellular niche. Recent findings have highlighted that reciprocal and dynamic interactions between the microenvironment, the stroma and the cancer greatly impact therapeutic responses. We therefore hypothesise that the multiparametric integration of these perturbations will predict cancerous phenotypes. We have recently developed an isogenic mesothelioma model engineered to comprise the most prevalent patient mutations, including loss of function mutations of *BAP1* and *NF2*. This isogenic cellular model represents specific patient groups well [2]. The PhD project will take advantage of this unique cellular model and use high-content, and label-free imaging, to identify stratified cellular responses. We will design, implement and validate a deep learning pipeline to integrate numerical and image data from cellular perturbation and perturbation inputs, classify wild-type and stratified KO cells, and predict the drug response with single-cell resolution.

Research challenge

This project aims to address the critical challenge of deciphering the complex heterogeneity in cancer cells by overcoming limitations in current bioimaging techniques, integrating AI to analyse cellular behaviour dynamically and at a single-cell resolution.

Data & methodology

This project is structured around three key objectives

Objective 1: Develop an AI-driven imaging pipeline to stratify mesothelioma patient groups. This pipeline will integrate customised machine learning algorithms, including model-based deep learning [5-7] and transfer learning, to process high-resolution imaging data. The pipeline will automatically identify and quantify cellular features from confocal and label-free images, enabling the classification of cell types and states in response to various treatments.

Objective 2: Determine personalised responses to changes in the mesothelioma microenvironment and therapeutics. We will use the AI pipeline to assess how individual cell populations within a patient's tumour respond to different therapeutic agents, correlating these responses with patient-specific genetic and proteomic profiles.

Objective 3: Integrate proteomics and AI-based imaging analysis to identify potential therapeutic targets. This integrative approach will enable us to link molecular signatures with phenotypic characteristics captured through bioimaging, pinpointing potential targets for therapeutic intervention.

Pilot dataset: We have established an initial dataset comprising >1000 confocal images, covering three genotypes: Wild Type (WT), and two independently generated clones of BAP1 and NF2KO. This pilot dataset will serve as a foundation for developing the imaging pipeline in objective 1.

Imaging platforms: We will employ two main high-content plate-based imaging platforms currently used by the Hansen lab [4]. These comprise the confocal-based Opera Phenix Plus and the quantitative label-free imaging platform, the NanoLive, that readily provides large datasets, in order to explore cell-level perturbations and responses across genotypes. These platforms allow for high-contrast time-lapse videos with single-cell resolution. These directly allow for multi-parametric analysis of cellular responses. Mechanistic insights will be further examined by utilising the confocal-based Opera Phenix Plus. These advanced platforms are available within the IRR, and constitute methodologies currently used by the supervisor team [4-7].

Cell model: We recently generated an isogenic cellular model, that represents the disease well [2]. The cellular model now allows us to differentiate what makes a cancer cell different to a healthy cell, but importantly also how a disease that manifests as one, is caused by mutations in very different genes, opening up for developing personalized therapeutics. We will utilise our high content- imaging for multiparametric analysis, which allows us to build an AI-generated predictive model. Our ongoing preclinical drug characterisation includes >10 of the most promising drug candidates. These drugs further allow us exciting opportunities to challenge and refine our model. We will further complement and validate our findings experimentally, including by paired genetical targeting. Importantly, we are able to test and refine the model iteratively during project development.

Model validation: We have through iterative rounds of comparative proteomics, both from 2D and 3D cultures of the isogenic cellular model, and by genome editing, generated knockout derivatives of differentially expressed proteins. This expansion of our cellular model provides additional mechanistic insights, and data depth, that can now be integrated into the later parts of the project. It will allow us to provide validation for our AI-generated model, gain further mechanistic insights, and identification of therapeutic opportunities. In addition, we have characterised patient-derived cell lines, and we will additionally obtain primary organoids from Mesobank.uk. These patient-derived cellular models will be analysed in the later part of the project and further allow for validation of our model. Combined,

these cellular models retain clinical features and facilitate the discovery of precision medicine approaches.

Ethical considerations

Currently, pleural mesothelioma is a deadly disease, where patients in general die within 12-18 months. As such the ethical implications of identifying potential stratified patient treatments are limited. However, if our approach enables early detection of the disease, then considerations into knowing this, for the in general elderly individuals, must be considered. Noteworthy, a clear benefit would be, that potential treatment options might be available, if the cancer is identified early. At the later stage of the project, we have the capacity to implement the use of patient-derived organoids from the UK-based Mesobank, for which Dr Hansen has ethical approval in place. We do not seek to carry out genome editing on primary cells.

Expected outcomes and Impact

The synergies in this project are centred around machine learning, high-content and label-free imaging, and a unique mechanistic clinically relevant cellular model. This combination provides an excellent opportunity to obtain transformative insights and new approaches in targeting mesothelioma. Although our focus is on mesothelioma, we expect to have a wider impact on cancer in general, such as initially in other cancers with either *NF2*, or *BAP1* mutated. Anticipated outcomes include: **1)** discovery of new therapeutic targets for mesothelioma; **2)** development of an AI predictive model, allowing for stratification of patients; **3)** a platform that enables preclinical drug discovery; **4)** the training of a T-shaped researcher with advanced interdisciplinary skillsets.

Training opportunities

This project offers comprehensive interdisciplinary training at the intersection of AI and biomedicine, focusing on impactful discoveries using AI-driven imaging techniques. The candidate will develop expertise in machine learning for biomedical image analysis, genome editing, high-content and quantitative label-free imaging, molecular biology, tissue culture, and programming with Python/Matlab. Additionally, they will engage with patient interest groups and attend professional development workshops provided by the Institute for Academic Development (IAD). The collaborative environment of the IRR (CGH) and the Institute for Imaging, Data and Communications (IDCOM) at the School of Engineering ensures a team-focused, inclusive culture, preparing the candidate to become agile researchers with a unique and comprehensive skillset essential for a successful scientific career. For more information about our teams, visit Hansen Lab and Yang Research Group.

References

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