WHITE PAPER

Ex vivo functional drug testing of 3D patient-derived cancer cells shows promise for personalized medicine.

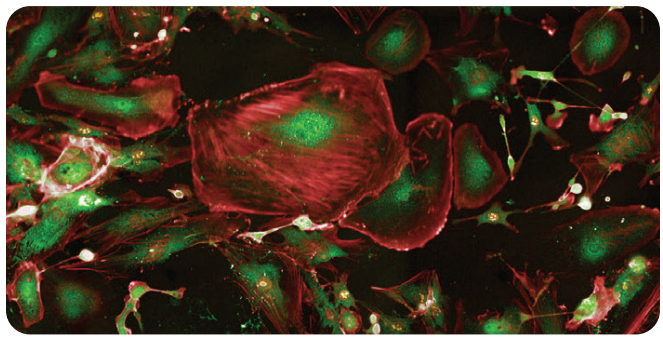
Introduction

It is widely recognized that no two cancers are the same and that each patient responds differently to treatment. However, recent reports of precision medicine trials demonstrating patient benefit have been disappointingly low. In order to deliver more personalized treatments, researchers need to look beyond pure genomics processes to a multi-dimensional assessment utilizing both genomic and image-based diagnostics.

Researchers at the Institute for Molecular Medicine Finland (FIMM), HiLIFE, University of Helsinki are demonstrating how functional screening of 3D patient-derived cancer cells enables effective drug sensitivity testing and the ability to potentially tailor medicines to an individual patient. Here, we speak to **Senior Scientist Vilja Pietiäinen and Principal Investigator Lassi Paavolainen**, both from FIMM, to hear more about their functional drug screening approach and how they are using imaging, AI, and machine learning models to uncover complex information from cancer cell and tissue samples.

Addressing the challenge

Over the last decade we have seen considerable progress in our understanding of the underlying causes of many cancers. This has led to a rise in targeted treatment approaches, which exploit the specific molecules and pathways involved in the growth, progression, and spread of cancer. These discoveries have led to major therapeutic breakthroughs in cancers that were historically considered difficult or impossible to treat and have led to significant advances also in precision medicine.



Despite the initial success of these therapies, many patients with advanced cancers become resistant to treatment. This can be explained by multiple factors including the effect of the tumor microenvironment and intratumor heterogeneity, which is one of the leading causes of resistance and treatment failure and one of the main reasons for poor overall survival in cancer patients with metastatic disease.

To address this challenge, a team of researchers at FIMM are using microscopic image-based solutions to investigate the response of 3D patient-derived cancer cells to a customized panel of anti-cancer compounds. The aim of this approach is to gain a better understanding of the cellular heterogeneity in cancer and elucidate an individual's sensitivity and resistance to different therapies. "Our aim is to characterize the phenotypic alterations or actions of the small molecules at the single cell level," explained Pietiäinen. "These might be morphological changes, or they might be changes in the localization of a certain protein. By profiling these perturbations in the cells, we can get a better overview of how these drugs are acting." These findings can then be used to advise clinicians on how to individualize cancer treatment, decreasing the risk of adverse effects and development of resistance to therapy.

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Functional drug sensitivity testing

Pietiäinen described how functional drug testing involves testing a panel of drugs directly on patient-derived cancer cells *ex vivo*, and then imaging and analyzing the phenotypic alterations caused by these compounds. Fresh tissue samples are first collected from the clinic, and then instantly processed and cultured. Once they have enough cells, they are perturbed with freshly made drug plates. The cells are then stained and imaged, features extracted, and the data analyzed. "This process means we can assess what happens at the single cell level," said Pietiäinen, noting that this is significant due to the heterogeneous nature of the disease. "What happens in one cell may not happen in another cell, which is important regarding further resistance of these cancer cells to certain drugs," she affirmed.

During the first stages of their experiments, Pietiäinen and the team must ensure that they have a representative cell culture. "We always need to validate the cell culture, and we do this by both phenotypic screening and exon sequencing," she said. "The cell model has to be compared to the original tumor to make sure we have a representative cell culture of that particular patient."

Another challenge is balancing throughput demands with the requirement to ensure assay relevance. Although 3D models are more physiologically relevant and predictive of drug response than 2D models, it can often be challenging to use 3D cell cultures in a high-throughput system. "Depending on the sample, we might use 2D for wider screens and then validate this with a smaller setup in 3D," explained Pietiäinen. "Sometimes there's only a small amount of the tissue sample to start with so we test in a smaller set of drugs first, which is defined by the clinician, and then if we are able to grow the cells for longer and they are still representative to the original tumor they are derived from, we can screen more drugs."

For the image-based cell profiling, which involves image acquisition with high-throughput microscopy systems and subsequent image processing and analysis, there are four key stages: feature extraction, data preparation, profiling, and analysis. Paavolainen explained that they utilize AI and machine learning models during this process to help quantify the treatment effects on the cells. "Compared to traditional drug screening assays, we take a more data driven approach where you collect lots of information and then try to mine the data to identify patterns that you maybe haven't seen before," he said. "These models can help analyze the data more robustly in an unbiased way."

The researchers note that quality control processes are required to ensure compliant and reproducible readouts from the screen. "This enables us, for example, to read the cell amounts from the image-based analysis. We then get quality scores for our screen so that we know that it has been successful," said Pietiäinen.

Depending on the cell culture, the whole process takes the team between two and six weeks. However, there is a strong emphasis from the clinicians for results to be delivered within one month. "There's no clinical relevance for most of the cases if we grow the organoids for half a year and then do the drug testing," said Pietiäinen. "We may have a beautiful, physiologically relevant model for biological discoveries, but it would be too late for the patient with a relapse cancer."

Developments in the field

Pietiäinen and Paavolainen both emphasize how advancements in technologies over the last five years have greatly facilitated their work. "Machine learning and AI were mainly considered research topics before, but now they can produce real, practical solutions which has changed how images are being analyzed," said Paavolainen. "Nowadays, basic analysis like segmentation of cells in 2D cultures can be done with pre-trained machine learning models. You don't need to tweak any algorithms or parameters to have an optimal answer. It has also improved the accuracy of our analysis."

For Pietiäinen, the ability to go to the single cell level has been a key enabler of productivity, especially the capability to combine different omics methods. She also notes that technological advances have meant they can work with more physiologically relevant cell cultures, which are better model systems for drug testing and cancer research.

COMPASS and the future

The team is currently part of the ERA PerMed-funded project on clinical implementation of multidimensional phenotypical drug sensitivities in pediatric precision oncology (COMPASS). One of the goals of the project is to build an international, standardized, and validated platform for drug testing based on image analysis and accompanying molecular analysis that characterizes and classifies different types of tumors for their response to different drugs. "To my knowledge, this project is the first of its kind where a large group of clinicians and researchers are working together from different countries," said Pietiäinen. "The main focus is to do the drug testing on pediatric solid cancers in different countries using the same drug plates and protocols. We're really aiming towards standardized drug testing for solid tumors."

Looking forward, Paavolainen expects to see the introduction of more unbiased pre-trained models that can extract information directly from the images. For example, the JUMP-Cell Painting Consortium, which is a collaboration between the Carpenter Lab, the Genetic Perturbation Platform, and the Center for the Development of Therapeutics at the Broad Institute of MIT and Harvard, along with industry and non-profit partners, is creating a public cell painting dataset to guide small molecules' progression to the clinic. "This is a good example of an openly available large data source for the machine learning community to learn from," he said.

Pietiäinen hopes to see the development of complex co-culture models, for example organoids with immune cells and other stromal cells. However, she notes that this approach comes with its own challenges, for example accurate segmentation. "You have to make a compromise between the microscope power, the throughput, and the physiological relevance and how deep you need to get. That is the real challenge here." She also believes that the future of image-based diagnostics lies in rare and inherited diseases, where morphological or functional changes due to a specific inherited mutation can be explored.

Conclusion

Considering the high level of heterogeneity of cancer and the fact that many patients do not respond or become resistant to standard therapies, it is necessary to take a more personalized approach to cancer treatment. Testing tumor cells from individual patients for sensitivity and resistance against anticancer compounds could help clinicians determine which therapy a patient is most likely to respond to.

State-of-the-art approaches, such as the drug sensitivity testing and image-based analysis of patient-derived tumor tissue and cells being conducted by the team at FIMM, can provide clinicians with a more comprehensive picture of an individual's cancer and potentially improve outcomes for patients that have already exploited other treatment options.

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About the scientists:

Lassi Paavolainen

PhD, Academy of Finland Fellow

Dr. Paavolainen is Principal Investigator at the Institute for Molecular Medicine Finland (FIMM), HiLIFE, University of Helsinki. His research focuses on uncovering complex information from bioimages using machine learning. His recently established research group is interested in developing novel deep learning solutions for bioimage analysis, studying various learning approaches to create general models, and applying these methods and models to profile cancer cell and tissue samples imaged using fluorescence microscopy.

Dr. Paavolainen obtained his PhD in 2013 from University of Jyväskylä focusing on microscopy image analysis research and software development. In 2015 he joined FIMM as a postdoctoral researcher in Horvath and Kallioniemi research groups where he focused on high-content image analysis of patient-derived cancer cells. In 2017, he co-created FIMM High-Content Imaging and Analysis core unit (FIMM-HCA) and worked as the first Head of the Unit. In addition to research activities, Dr. Paavolainen is President of the CytoData Society, and Information Officer in the board of Nordic Microscopy Society.

Vilja Pietiäinen

PhD in Virology, Docent; Adj. Prof. in Cell and Molecular Biology

Vilja Pietiäinen is a team leader and senior researcher at FIMM, with expertise in cell biology, precision medicine, patient-derived 2D and 3D cell models, and high-content microscopy. She is also a co-director and co-founder of FIMM High Content Imaging and Analysis Core unit. She has led and managed several high-content imaging related projects at FIMM e.g., in EU FP7 on Systems Microscopy, industrial collaborations, Era PERMED COMPASS project for standardized drug testing of pediatric solid tumors, and iCAN Flagship subprojects.

Her key interests are to explore the cancer pathogenesis and improve cancer therapies for functional precision cancer medicine. This is mainly carried out by studying and combining functional phenotypic properties of patient cancer tissues and advanced personalized primary cancer cell models with other omics and drug testing data. During the COVID-19 pandemic, her team has also, in collaboration with virologists, established assays for identifying antivirals and antigen responses against SARS-CoV-2.

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