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Human lung-on-a-chip technology used to study behavior, drug responses of lung cancer in its natural environment

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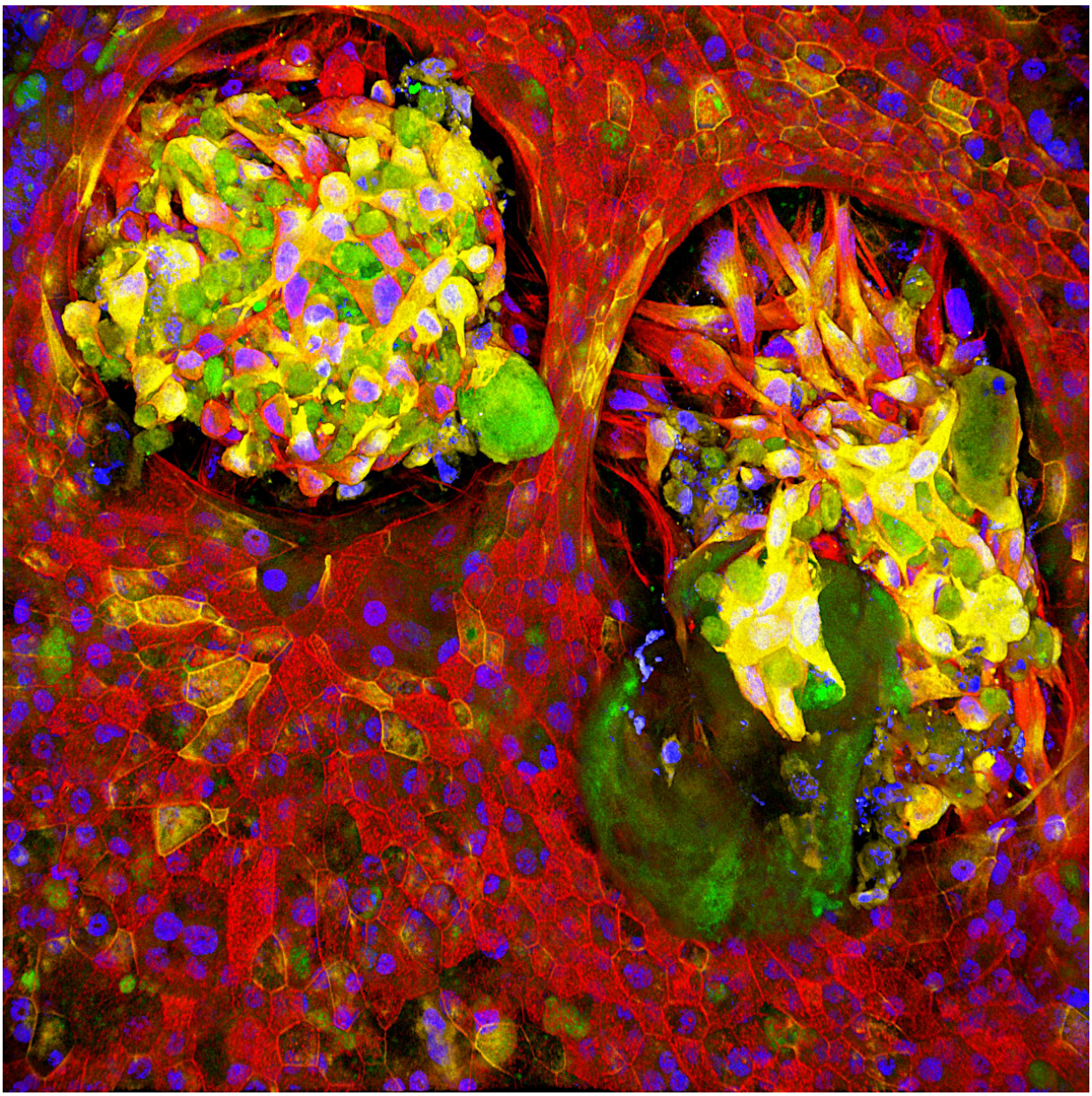

This image shows how NSCLC adenocarcinoma cells can be grown as a tumor cell colony (yellow) next to normal human lung small airway cells (red) in the lung epithelial channel of the Lung Cancer Chip. Credit: Wyss Institute at Harvard University

Cancer researchers have come to understand that generating human tumors in mice by injecting cancer cell lines under the skin does not recapitulate how tumors normally emerge and spread to specific organs in the human body, nor how they respond to anti-cancer drugs. So, they turned to injecting tumor cells at the organ sites where they originated from in humans, so-called 'orthotopic' sites. Orthotopic tumors, such as those created by injecting breast cancers into the mammary fat pads of mice, exhibit growth and metastatic behaviors more like those seen in patients, however, these organ environments are still not human. It is also not possible to visualize how tumor cells grow, move and respond to therapeutics in these orthotopic animal models, which restricts our ability to understand how different organ microenvironments influence tumor behavior and thereby develop better drugs.

As reported in *Cell Reports*, a team at the Wyss Institute for Biologically Inspired Engineering led by Founding Director and Wyss Core Faculty member Donald Ingber now has leveraged its human Organs-on-Chips technology to confront this challenge. In previous work, the team successfully modelled two different regions of the lung—the air-conducting small airway and the oxygen and carbon dioxide-exchanging alveoli at the tips of the small airways—in microfluidic devices that are created with microchip manufacturing methods. The different lung cells inhabit one of two microchannels that run parallel through the chip, separated by a thin porous membrane from a microvessel lined by

human lung endothelium in the second channel.

Like in the human lung, the resulting small airway epithelium is thicker, stiffer and covered with moving cilia, while the thinner alveolar epithelium is more permeable to enable efficient gas exchange and it is exposed to cyclic mechanical deformations to mimic breathing motions in the chip. The researchers continuously stream cell culture medium through the vascular channel to support the epithelial and endothelial cell layers over many weeks as occurs with blood-flow in living lung. In addition to having engineered the basic tissue architecture and functionalities of these two lung regions on chips, the team previously showed that they can successfully model lung diseases, including chronic obstructive pulmonary disease (COPD), asthma and pulmonary edema.

In this new study, the team developed human orthotopic lung cancer models using these two lung chips. Approximately 85% of all lung cancers are diagnosed as non-small cell lung cancer (NSCLC), and the team focused on the adenocarcinoma form of this cancer which roughly accounts for 40% of all NSCLCs. In the human body, NSCLC adenocarcinoma cells are known to arise at the interface between the human lung's small airways and alveoli. But the tumor then primarily grows within the alveolar structures.

Ingber's team showed that when NSCLC adenocarcinoma cells are grown in the Lung Airway and Alveolus Chips, the tumor cells grow rampantly in the microengineered alveolar microenvironment whereas they remain quiescent in the Airway Chip, just as is observed in human patients. "Our lung cancer-on-chip platforms can model central aspects of orthotopic NSCLC in real time and high-resolution, and much more closely than other in vivo and in vitro approaches. They offer a literal window on the biological tumor complexities," said Ingber, M.D., Ph.D. Ingber also is the Judah Folkman Professor of Vascular Biology at HMS and the Vascular Biology Program at Boston Children's Hospital, as well

as Professor of Bioengineering at the Harvard John A. Paulson School of Engineering and Applied Sciences (SEAS).

To achieve this, the researchers developed co-plating and injection strategies that enabled them to stably integrate a small number of NSCLC cells into the two lung chips. "This approach allows us to recreate key hallmarks of this cancer, including its growth and invasion patterns, and to determine how they are influenced by cues from surrounding normal cells. In the Airway Cancer Chips, cancer cells remain dormant for up to 12 days before they started to grow, while in Alveolar Cancer Chips they commence their growth much more rapidly, and once they reach a critical mass, they separate themselves and invade the endothelium as part of their metastatic process," said first author Bryan Hassell, Ph.D., who as a SEAS Graduate Student on Ingber's team developed the lung cancer-on-a-chip platform.

The team next asked whether physiological breathing motions within alveoli might affect cancer cell behaviors. Suprisingly, they discovered that when cyclic mechanical forces are applied to the lung epithelial channel to mimic breathing motions, both cancer cell growth and invasion were potently inhibited. "This is the first time that a clear impact of cyclical breathing motions on cancer cell growth and invasion has been demonstrated in any in vitro system modeling NCSLC behaviors," said Hassell. The researchers think that when lung cancer cells grow and fill patients' lung alveoli, this interferes with their natural motions, which in turn could speed up tumor growth and facilitate invasive behavior.

Finally, they took their approach another step further by investigating whether breathing mechanics could affect the sensitivity of NSCLC cells to a clinically used anti-cancer drug, known as a tyrosine kinase inhibitor (TKI). TKIs target frequently mutated enzymes, like the so-called epidermal growth factor receptor (EGFR), which unleashes unfettered

growth in NSCLC and other cancers. Because early-generation TKIs can lose their efficiencies when cancer cells become resistant to them by producing new EGFR variants, cancer researchers are trying to design ever smarter TKIs. However, the constantly morphing cancer cells learn to deal even with those, by genetically rewiring themselves so that they can activate alternative cancer mechanisms.

Importantly, Ingber's team found, that in the Alveolar Cancer Chip, NSCLC cells that are already resistant against a first-generation TKI, can still be stopped cold in their tracks by a third-generation TKI in the absence of breathing motions, as might occur within large tumors that fill the lung's alveoli and stops their motion. However, in breathing mode, they become impervious to the drug and continue to survive and grow slowly, essentially creating cancer 'persister' cells that are known to be the nemesis of cancer therapy.

The researchers also observed that the levels of cytokines, proteins involved in cell communication that are produced by epithelial and endothelial cells, as well as cancer cells, and known to be important prognostic indicators for NSCLC, reflect cancer growth in the Alveolus Cancer Chip and are modulated by breathing motions and drug treatment. "The effects of breathing motions on cancer cell behavior in our models could explain how tumor cells, which remain from a shrinking tumor after therapy, could become persister cells, able to defy drug therapy, linger and eventually cause the cancer to relapse. Our orthotopic in vitro platform thus could be well-suited for dissecting how these persister cells arise, and they may be a useful tool in future drug development efforts that aim to eradicate them," said Ingber.

Provided by Harvard University

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