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Prof. dr. I.H. Heijink

Let's Keep in Touch

Strong Cell-cell Contacts for Healthy Lungs

Inaugural lecture

6 October 2023

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Inaugural lecture by

Prof. dr. I.H. Heijink

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Members of the Executive Board,
highly valued attendees,
dear friends and family,

Let's keep in touch. Today I will talk to you about my research into the origins of lung diseases, and the important role I attribute to connections between certain cells.

Function of our lungs

To understand my research, it is first of all useful to know why we have lungs. You are probably all familiar with the function of our lungs, but how exactly do they work (Figure 1)?

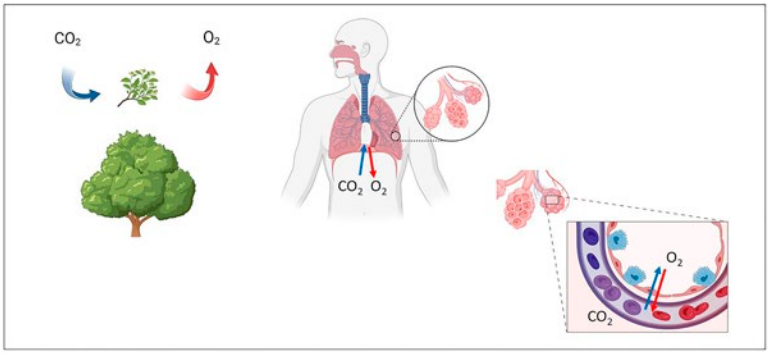

Figure 1. The function of our lungs.

Deep within the lungs are the alveoli, responsible for gas exchange. Oxygen (O2) is taken up from the air and delivered into the blood and organs. Here it is consumed, and the waste product co2 is again removed from the body by the lungs. This function can be compared to the function of the leaves in a tree; these convert co2 into oxygen through photosynthesis and release it into their environment. Oxygen is used in the building blocks of our organs, the cells, to convert nutrients into

energy. Small organs in the cell called organelles are responsible for this. These organelles are referred to as mitochondria, also known as the energy factories of the cell (Figure 2).

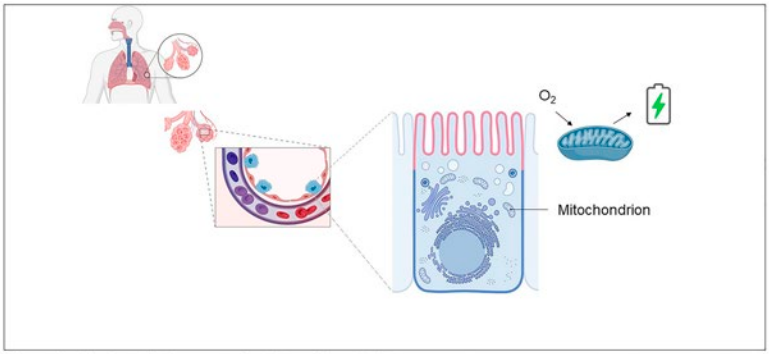

Figure 2. Mitochondria: the energy factories of the cell that consume oxygen.

Mitochondria – The energy factories that consume oxygen

According to an old theory, our cells have incorporated these mitochondria at an early stage - as unicellular organisms - very early in evolution (Figure 3). Until then mitochondria functioned independently as a simple, prokaryotic organism. This theory dates back to 1883. A few decennia later, the first experimental evidence was found to support this hypothesis. This was done by an American biologist, Professor Ivan Wallin. He was known for his eccentric behavior and throwing parties for his students with a lot of booze; apparently, they have always been fun people, those biologists. Wallin was also very productive as a researcher, publishing a series of papers on the nature

of mitochondria from 1924 onwards. He and others proposed that the uptake of the mitochondria enables cells to convert nutrients into energy. This is done through oxidation, where energy-rich electrons are extracted from nutrients with the support of oxygen, which then enables many processes in the cell, such as cell division and protein production.

Let's take a small leap in time, about 2 billion years, during which unicellular organisms evolved into complex organisms consisting of multiple organs, all of which have to be supplied with oxygen in order for biological processes to proceed and the internal environment to be maintained. This required the development of a specialized organ. In other words, and to quote a pulmonologist colleague: without mitochondria we would not need lungs.

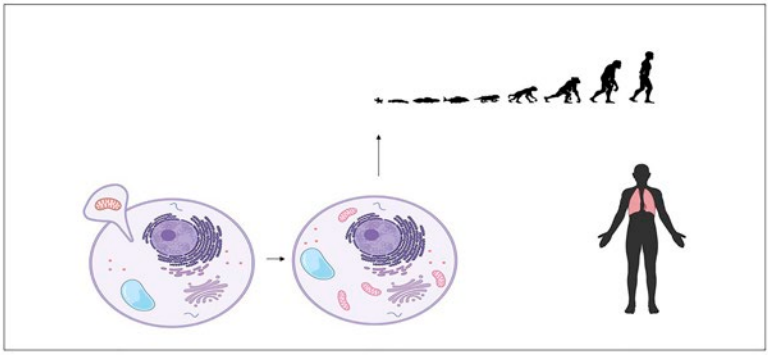

Figure 3. Incorporation of mitochondria in eukaryotic cells (cells containing a real nucleus) during evolution.

The epithelium: Protection from the inhaled world

The oxygen we breathe first enters the airways, also called bronchi, which have many branches and conduct the oxygen to the alveoli (Figure 4).

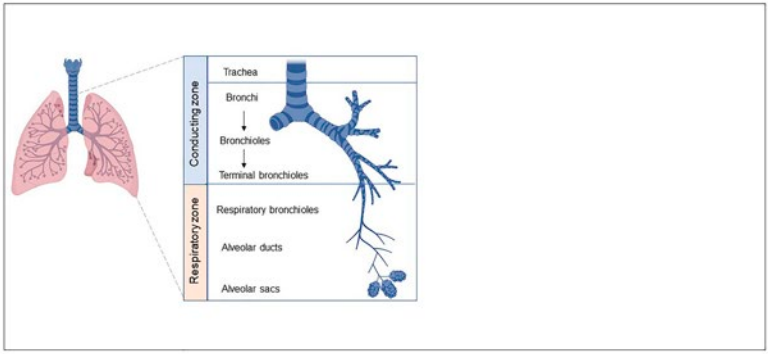

Figure 4. The structure of the respiratory tree.

These alveoli have an enormous surface. In comparison, if you spread out all the alveoli, you could cover the entire wall of a squash court. Because the lungs are in direct contact with the outside world, the lung is an extremely vulnerable organ. Especially in the times we live in now, with climate problems, increasing air pollution, exhaust gases, PFAS and other harmful substances (Figure 5). But these are not the only challenges our lungs face; pathogenic bacteria, viruses and fungi also pose a major threat to our internal environment. The consequences of infection can be tremendous and can lead to life-threatening and long-term damage to the lungs, which has become painfully apparent during the past pandemic.

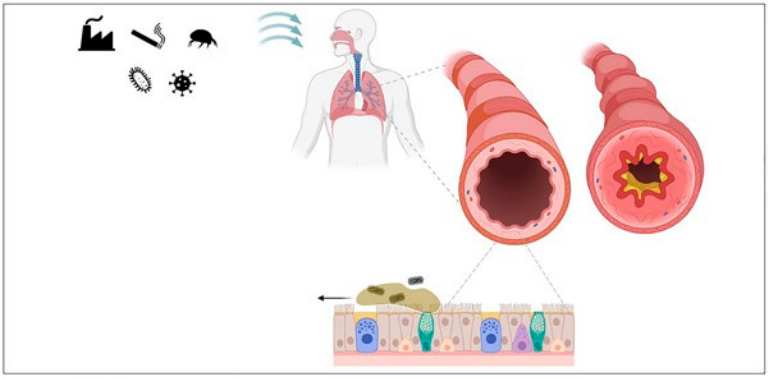

Figure 5. Epihelium: protecting the lungs from the inhaled environment.

The lungs have a very sophisticated defense mechanism against the damage from the outside world. The smooth muscle cells that circumvent the airways can contract to cause a temporary constriction when harmful substances from the external environment are inhaled. From the nose to the lungs, the airways are lined with the so-called epithelium or mucosa. In the airways, the epithelium consists of cells that can produce mucus, in which bacteria and other foreign particles can be trapped, which can then be removed with the movement of the cilia on neighboring epithelial cells, and finally be coughed up.

In addition, the epithelium provides a physical barrier, enabled by the connections formed between neighboring cells. This is done by specialized molecules that act as a kind of zipper that selectively lets substances through (Figure 6). The molecules most at the top of the connections are referred to as tight junc-

tions. These make the epithelium impermeable to harmful substances. Located underneath are the connections we call adherens junctions. Here, the molecule E-cadherin has an important role. You could think of E-cadherin as the start of a zipper, allowing the formation of all other contacts between the epithelial cells.

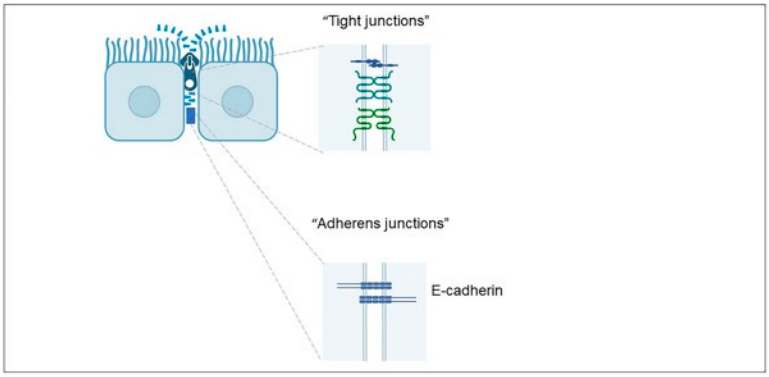

Figure 6. Epithelial cell-cell contacts.

Also, like cells of the immune system, the epithelium is capable of recognizing pathogens, albeit in a non-specific manner, being part of the innate immune system. When epithelial cells sense damage or harmful microbes, they can secrete antimicrobial proteins. In addition, they can secrete substances that act as alarm signals and call upon inflammatory cells, including white blood cells (Figure 7). Cells of the acquired immune system can then be activated to produce antibodies or to develop into attacking cells that help to clear the pathogens. The epithelium of the nose and

lower airways can also support in the production of specific antibodies (Immunoglobulin A) that protect against pathogens and are secreted in the mucosa. Nasal immunization has therefore been suggested as a good defense strategy against respiratory viruses, especially because this is the site of entrance of the viruses from which they spread. There are some tricky parts to this, for example we need to get the medicine across the mucus layer, but I think it will be good to put more effort into this, so that in future pandemics it would be easier to immunize in the nose. I expect that this will meet with less resistance than injection into the muscles, people don't seem to have that much trouble putting all kinds of stuff up their noses.

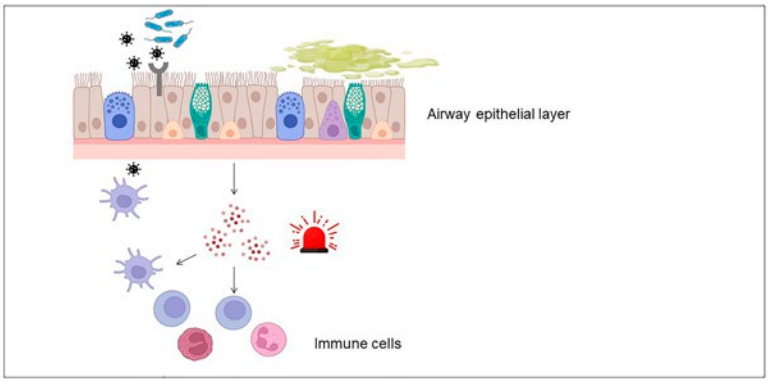

Figure 7. Immunological role of the epihelial barrier.

Epithelial damage and lung disease

The epithelial layer also provides protection against allergy-inducing substances such as house dust mites, and toxins such as

tobacco smoke. Like viruses, these can disrupt contacts between neighboring cells. My research from the time I worked in Edo Vellenga's lab, published in 2007, showed that loss of epithelial cell-cell connections leads to increased production of alarm signals for specific immune cells. Loss of cell-cell connections can also make the cells more vulnerable and more likely to be damaged or even die and subsequently start to emit alarm signals (Figure 8). We have demonstrated this in various articles published over the past years through the research of my former students and PhD students Sijranke Post, Virinchi Kuchibhotla, Kingsley Nwozor and XinZi Zheng, who I supervised together with Martijn Nawijn, Corry-Anke Brandsma, Tillie Hackett from Vancouver and Alen Faiz from Sydney, in the lab supported by the technicians Jacobien Noordhoek, Harold de Bruin, Marnix Jonker and Marissa Wisman.

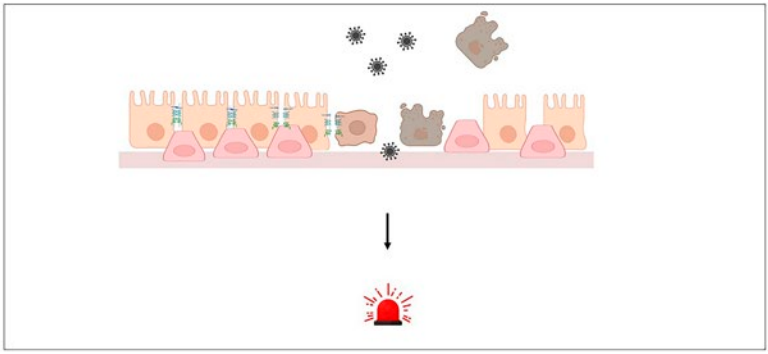

Figure 8. Damage of the airway epithelial layer.

The alarm signals secreted by the epithelium are therefore crucial for stimulating the immune system and breaking tolerance

to certain substances. The extent to which the epithelium is damaged could well be a determining factor in whether or not an allergic reaction develops. Also upon cigarette smoke inhalation, damage to the epithelium probably plays a similar role in whether or not chronic inflammation develops in the lungs. The type of irritating substance determines what type of inflammation develops. The immune cells that invade the lungs then have to clear the invader, and the tools they use for this usually cause collateral damage and further harm the epithelium.

Normally, the epithelium regenerates and forms a functional layer with beating cilia again, allowing the epithelium to suppress instead of propagate inflammation (Figure 9).

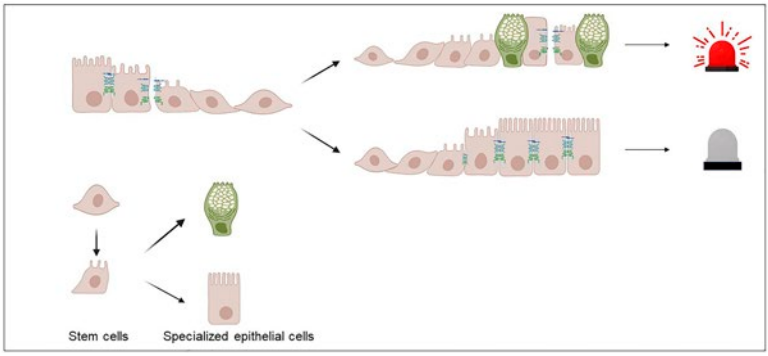

Figure 9. Airway epithelial repair.

For the repair of the epithelium after damage, progenitor cells (or stem cells) present in the lungs first have to proliferate and then specialize into well-functioning mucus and ciliated epithelium.

This specialization requires that the cells form proper connections with their neighbors again. If epithelial cells cannot recover properly, more mucus-producing cells can develop. The cells can also remain in a state where they continue to produce an excess of alarm signals, screaming for help. Disease can develop (Figure 10).

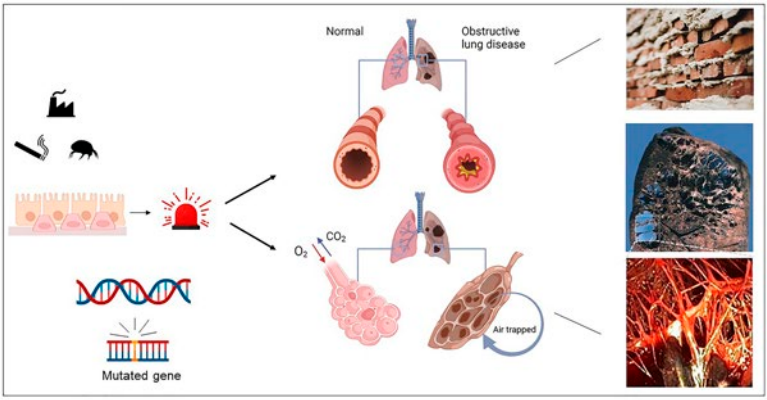

Figure 10. Epithelial damage and lung disease.

In addition to inflammation, certain of these substances can stimulate underlying cells to temporarily pave the damaged area by the production of structural protein fibers. This can for instance be compared to the formation of scar tissue after a wound in the skin or repairing a wall with cement in instead of brick. You can imagine that when this happens in your airways, it becomes much more difficult to breathe.

Furthermore, in the case of smoking, where the irritating substances penetrate deeper into the lungs, the alveoli can also be affected by the inflammation. As a result, the tissue is destructed, elastic recoil is lost and large holes can form. While air can still enter, gas with CO_2 is no longer removed, causing severe breathing difficulties. For the recovery of the alveolar epithelium, it is necessary that the surrounding cells (stromal cells), including a certain type of stem cell (mesenchymal stromal/stem cell), produce growth factors. The research of my team has suggested that the interaction with surrounding cells may be impaired in COPD and thus renewal of the alveolar epithelium is not adequately supported.

Susceptibility to develop lung diseases

Collectively, this shows that damage to the epithelium plays an important role in lung diseases. Yet what determines the extent to which the epithelium can repair itself or, conversely, remains damaged? Why is one person sensitive to this and another not, and why do some people develop asthma while others are not susceptible to allergic stimuli? Why do only 20-25% of smokers develop COPD?

In addition to the various exposures that we incur during our lives, personal factors are involved, such as age, sex, body weight, nutrition and also hereditary predisposition.

Although lung diseases such as asthma, COPD and pulmonary fibrosis do not involve an error in one specific gene, many genes have been identified in recent decades in which inter-individual variations may contribute to susceptibility to developing disease. Remarkably, the transcribed products of these genes (the proteins) are often expressed in the epithelium of the lungs, which once again underlines the importance of the epithelium in the pathogenesis of these diseases. It is often still unclear how exactly these genes may contribute to the sensitivity to external stimuli, but several of the susceptibility genes have been linked to how the epithelial cell deals with damage. An example of this is the research into the gene FAM13A by my PhD student Qing Chen, which I was privileged to supervise with late professor Marike Boezen and Maaike de Vries. We showed that this gene is involved in recovery of the epithelial barrier and dealing with oxidative stress upon smoke exposure.

Not only changes in genes themselves determine the personal way in which our cells behave, but also changes in factors that are located *on* the DNA (*epi*genetic factors) and are influenced by the environment; for instance, very small pieces of genetic material, microRNAs. My PhD students Emmanuel Osei, Hataitip Tasena and Mirjam Roffel investigated this within a European collaboration with, among others, the University of Ghent. They found differences in the epithelium of healthy people and patients with asthma or COPD.

Also, the effects of smoke and other toxins on the mitochondria may contribute to the heterogeneity between individuals. The radical substances in smoke can damage the DNA of the mitochondria. This can then lead to more formation of reactive oxygen species and oxidative stress, especially in the case of long-term damage or when antioxidant levels are insufficient. This causes stress within the cells and promotes the production of alarm substances. In addition, oxidative stress causes cell aging and this can lead to the exhaustion of the regenerative capacity of stem cells.

My PhD supervisor Henk Kauffman already raised the enthusiasm for mitochondria in is team at the beginning of the millennium, but it was not until 2013 that we were among the first to publish about the abnormality of mitochondria in the epithelium of COPD patients (Figure 11). This discovery was made by former PhD student Roland Hoffmann, who I supervised together with Antoon van Oosterhout. Here, we worked closely with researchers Schols, Langen and Gosker from the NUTRIM school for nutrition and translational research in Maastricht.

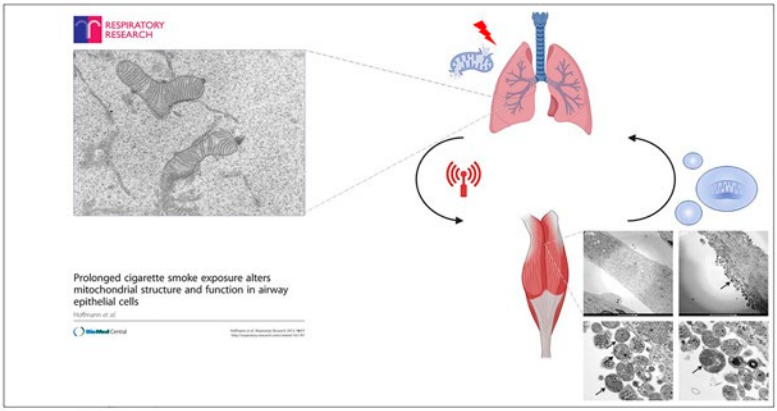

Figure 11. Mitochondial abnormalities in COPD.

Interaction between lungs and skeletal muscles

Mitochondrial health can be affected not only by the outside world but also by our internal milieu. In one of my current collaborations, we study the role of mitochondria in impaired lung epithelial repair as well as muscle tissue repair in COPD. We have an exciting hypothesis on the interaction between the two organs. Of course, we have known for a long time that a healthy diet and exercise help to maintain healthy lungs, and it has also been shown that exercise and training of the skeletal muscles can improve the function of the lungs in COPD patients. But why?

Skeletal muscles appear capable of sacrificing themselves at the expense of other organs and release certain substances that can promote tissue recovery in other organs. Excitingly, my Maastricht colleagues have shown that muscles can release

mitochondria into the circulation in small vesicles. Similarly, the work of Crewe and co-workers (published in Cell Metab. 33(9):1853-1868, 2021) has shown that other metabolic cells, fat cells, can release their mitochondria, which can then be absorbed by the heart. We are now investigating whether a similar process can contribute to repair of the epithelium in the lungs, and whether the weakened muscle in COPD transmits fewer mitochondria or more damaged mitochondria to contribute to impaired lung tissue repair in COPD. We use an organ-on-chip model that is being developed in the team of Sabeth Verpoorte. Studying this communication may open new avenues for treatment, for example through a combination of muscle exercise with certain nutrients and/or antioxidants.

Treatment of lung diseases

I have come to the point where I would like to emphasize the importance of finding novel treatment options for the various lung diseases. Lung diseases are often very debilitating for the patient. In addition, they are associated with high mortality. COPD is currently the 3rd leading cause of death worldwide (Figure 12).

A patient who I know well once told me that experiencing COPD is as having to climb a steep slope with every movement you make and everything you do. COPD is a disease that you have all of the time, you constantly need to regulate your respiration. You become more and more dependent of oxygen and care by

others. What bothers most is that the disease only worsens over time and that there is no cure.

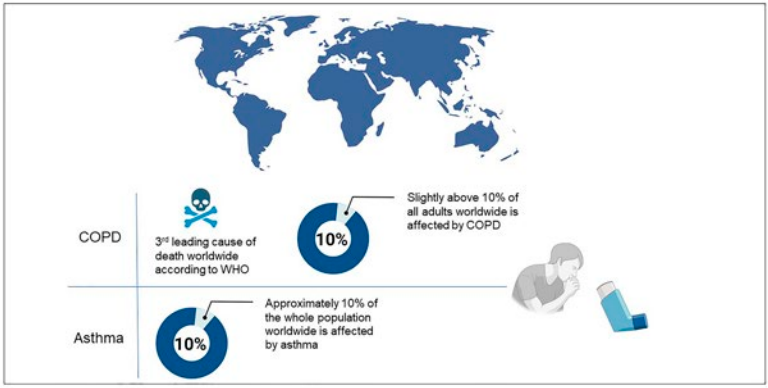

Figure 12. COPD and asthma epidemiology.

Asthma is also highly prevalent. One in 10 people suffer from the disease, and the prevalence is still increasing. Although the disease is reasonably under control here in the westernized world, it is often underdiagnosed and undertreated in low and middle income counties, and it still has a huge impact on the quality of life of patients, with a strong dependence on puffs.

At the moment there is no curative treatment for any of the diseases mentioned, and at best the symptoms are temporarily controlled. There are new developments such as biologicals, but they do not always address the cause. In my view, this is very important, and this is why I see the epithelium as a promising target. It is crucial to understand why the epithelium

becomes damaged in lung disease and how epithelial repair can be improved. The more the tissue is damaged, the more difficult it will be to revitalize its repair. Usually, the damage goes unnoticed for a long time before the disease is diagnosed. It is therefore vital to detect the damage earlier.

Furthermore, we still do not fully understand which underlying mechanisms are responsible. I have already mentioned that personal factors contribute to respiratory disease, and there is a wide variety in how our lungs deal with harmful substances. Not only between different diseases, but also within diseases. For example, one person with COPD may suffer from phlegm and coughing, while another has emphysema of the lungs in part of the lungs, and yet in another person the entire lungs affected with loss of elastic recoil. A person can develop asthma at a young age, which is often caused by an allergen such as house dust mite, while asthma can also develop later in life, which is more often caused by inflammation related to smoking or other toxins.

Because of these different underlying mechanisms, a personalized approach is required, and not one size fits all. For precision medicine it is important to find better biological indicators (biomarkers) to know what type of underlying disease a patient is suffering from and also to what type of treatment a patient will respond best. This requires large-scale studies and collaboration of many institutions to collect and combine extensive data

and materials from patients, using innovative technologies and big data analysis.

Furthermore, patient-specific models are needed to find new mechanisms and identify novel targets for treatment. This is challenging, especially because the lung has such a complex structure. There are many different cell types, each with their own specialization, yet with strong influence on each other. The surrounding protein fibers also instruct the behavior of the cells, as is clear from the research of my colleague Janette Burgess and as exemplified in the lab by Mehmet Nizamoglu and Taco Koster. That is why we need advanced culture models that more closely mimic the three-dimensional structure of the lungs and all its different interacting components (Figure 13).

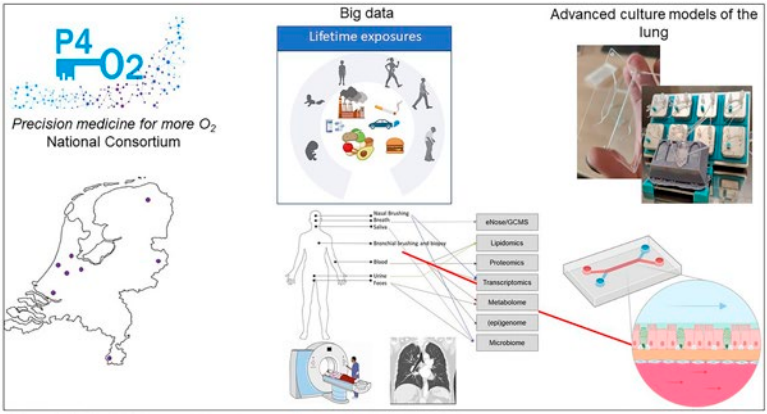

Figure 13. The P4O2 consortium program.

Novel treatment targets for lung diseases

This is all possible because of multidisciplinary collaborations within our research institute GRIAC (Groningen Research Institute for Asthma and COPD) as well as within the Netherlands in our current research program P4O2 (precision medicine for more oxygen). This is a large national consortium led by Anke-Hilse Maitland-van der Zee of the AMC. Through collaboration with multiple clinical centers, we collect a wide variety of samples from the whole body of patients with disease and individuals at risk to develop disease, which we will correlate and link to environmental exposures. We are setting up a large COPD cohort at the UMCG, led by Maarten van den Berge and Dirk-Jan Slebos. Furthermore, I am in lead of developing advanced culture models with patient-specific cells. My team has built up a large biobank containing these cells over the years, a very valuable treasure. In addition to the aforementioned technicians, Frederique Alleblas has contributed to this since she replaced Leonie Apperloo in her position of research technician on the P4O2 project.

In an in-house developed chip model we emulate the 3D composition of the airways with blood flow and air exposure. I am co-supervising this project with one of the talented post-docs in our group, Daan Pouwels. PhD student Brady Rae has been working very productively on this. P4O2 post-doc Gwenda Vasse has been instrumental in refining the epithelium-air-in-

terface model, next to her own research on the susceptibility to develop lung damage in COVID-19.

In addition to exposures to the external environment, we aim to study the interaction of lung cells with the internal environment in these models. Ultimately, the lungs do not stand on themselves in the body, but are under mutual influence of the immune system and other organs. This communication can be disrupted in diseases such as COPD, as evidenced by the fact that COPD is often associated with other conditions such as muscle weakness, which I mentioned earlier, and also cardiovascular disease, osteoporosis and diabetes.

The patients involved in our P4O2 consortium have indicated that they find it important that we look into the interaction between organs. Patients do not wish to be seen and treated as an organ, but as a whole. Often not just one single disease is present, but several disorders at the same time that are related to each other.

The P4O2 project has been based on the national initiative to draw more attention to lung research and healthy lungs in the Netherlands – the National Program for Pulmonary Diseases. This was initiated under the leadership of Dirkje Postma (together with Peter Sterk), who has not only been very impor-

tant for my personal career, but in this way also for the entire lung research community in the Netherlands.

During one of the early meetings of this program, patients, pulmonologists and researchers brainstormed with each other and it emerged that one of the most important areas of focus for patients is to find a strategy that will enable tissue repair again.

Novel strategies targeting the epithelium

This is exactly what we aim to do in another large consortium that I lead, for which we recently received substantial funding (Figure 14). In healthy lungs, the epithelium has a remarkable ability to regenerate, for example when viral infection has caused damage. Our previous research has shown that this restorative capacity is not completely lost in patients with COPD. However, we propose that the stem cells and structural fibers in the nearby environment provide insufficient support for epithelial repair. We will use a new technology to improve the support within the microenvironment (the stromal niche), developed by Jeroen Leijten's team from the University of Twente. We aim to deliver stem cells or their growth factors to the nearby environment to revitalize regenerative responses. We will package the cells or growth substances separately in a kind of hydrogel, which enables them to reach the deeper lungs and to be retained here longer, which is currently not possible. Our approach is based on new insights obtained in the project of

PhD student Dennis Kruk, which I supervised together with Nick ten Hacken. Post-doc Lei Wang is now following up on this in her research project.

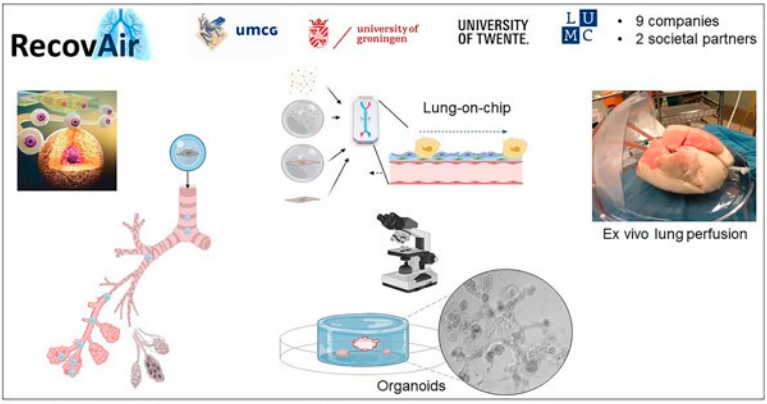

Figure 14. the RecovAir program.

We will test the effectiveness of the strategy in a lung-on-chip model from colleagues in Leiden (Pieter Hiemstra and Anne van der Does) and in an organoid model, in which we grow a kind of mini-lung in collaboration with regenerative pharmacologist Reinoud Gosens. This model has been optimized in my group by Marissa Wisman and Jacobien Noordhoek, and the model especially instrumental to follow how an epithelial layer can recover upon damage, re-organizing from a single cell into a 3D structure in which all the necessary cells are present. Finally, we also need to know how our strategy performs in a functional organ and we will test our strategy in a model where a full human lung breathes outside the body, called ex vivo lung

perfusion, together with a team of thoracic surgeons, including Michiel Erasmus, Tji Gan and Erik Verschuuren.

Societal impact of and beyond my research

This all sounds very exciting and promising, at least in my perception, but this research will take a long time. It will take at least 5 and potentially even 10 years before it can lead to a treatment. I feel it is important to be open about this and we as scientists should always do so. We should communicate clearly, do not promise too much and be transparent about pitfalls. The credibility of science has been increasingly challenged in past decades, and trust in science has been put to the test even more during the recent pandemic.

Explaining complicated research in clear language is challenging. Nevertheless, we can nowadays make use of supportive improvements, such as in Artificial Intelligence. The developments here are unprecedented and exciting. However, I feel that we should be well aware of the consequences of too strong interference of AI within our society. It is still difficult to oversee the risks and limitations, such as bias, and especially whether AI can pose a threat on education, because it limits the development of specific skills.

Science is invaluable to our society and the transmission of knowledge is one of the few not yet obsolete features that dis-

tinguishes us from other animals. A very grateful task for the university. Let's make sure we do not arouse suspicion, but transfer our knowledge in a reliable way. Let's educate our students with a good understanding of academic integrity, teach students from the beginning of their scientific career how to communicate their research results to society, while keeping the interests of patients and the social relevance of their research in mind.

The challenge is to not focus too much on the applicability of research. In the past, it has often been proven that scientific research where the final goal is not yet completely determined leads to major discoveries (for instance think of the discovery of penicillin as treatment for bacterial infections by Alexander Fleming in 1928). Foster creativity and open-mindedness through collaboration with other disciplines. Stimulate curiosity about the unknown, be supportive of research with an open end, where any economic benefit is not yet clear at first glance.

In addition, it would help avoid perverse incentives and lower the production bar for publications. There is increasing stress among students, which may be due to the high pressure to multiple various papers in a relatively short time. Furthermore, I believe this is in part also created by peer pressure. I am convinced that social media have a role, constantly having to live up to an image of perfection. There is high social pressure,

the telephone that needs constant attention. Moreover, we live in a world with many regulations and a lot of bureaucracy. This is stressful for our society. We will not be able to prevent all harm, and not every incident should lead to new rules and regulations. As for our students, we should teach them better how to deal with harmful influences that can also happen later in their later careers.

To get back to the topic of my research, we should also strive for a lung epithelium that is more resilient to harmful insults.

Nevertheless, prevention is important and we should do something about the air quality and the changing climate, for the health of our lungs and beyond, for the health of our world in general. It is becoming increasingly clear that for instance inhaled microplastics pose a threat to our respiratory system. At the same time, life in the oceans is also threatened by the surplus of (micro)plastics. Notably, life in the oceans actually stores a large share of CO_2 emissions, a major threat to the climate. Early in my lecture, I compared the lungs to trees that filter CO_2 from the air, but as pointed out by Esther Heijink, a well-recognized consultant in circular economy, the CO_2 storage capacity per whale is has been calculated to be equal to that of 3000 (!) trees. So, we should protect our oceans and its wildlife, including whales, much better. Changing our behavior to achieve this will not be not easy, especially as long as economic

growth, profit and overproduction remain the norm. We should find a way to something about this excessive production.

To conclude

If we can improve the contacts between the epithelium, we can make the lungs more resilient, hopefully enabling better health for our lungs. I will continue to work hard for this, supported by my team and all members of lab (Figure 15).

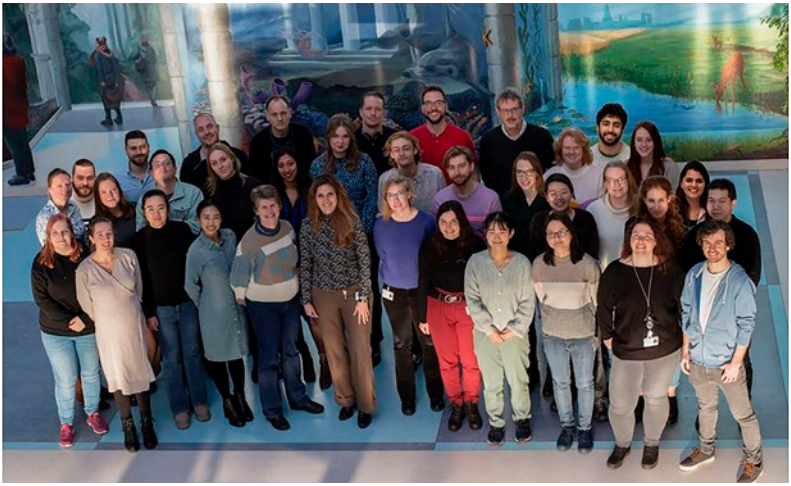

Figure 15. The EXPIRE team.

Acknowledgements - Teamwork

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Dixi - I have said

Irene Heijink (1975) was trained as Medical Biologist and obtained her PhD at the Faculty of Medicine in 2004. She is fascinated by the mechanisms underlying aberrant damage and repair of the first line of defense against the inhaled environment, the respiratory epithelium. She leads the Experimental Pulmonary and Inflammation research (EXPIRE) lab, and since 2022 she is program leader of the multidisciplinary and translational Groningen Research Institute for COPD (GRIAC). Irene Heijink is currently also head of the European Respiratory Society (ERS) Assembly Basic and Translational Sciences. She and her team study the role of the lung epithelium in the pathogenesis of lung diseases as asthma and COPD, using advanced patient-specific culture models. In her chair as professor of Cellular and Molecular Lung Pathology, she aims to develop new strategies to repair the epithelial barrier in lung diseases and thus halt or even reverse disease.