Extracellular vesicles as mediators of the response to respiratory exposures

IV – Valorisation

"The important thing is not to stop questioning. Curiosity has its own reason for existing. One cannot help but be in awe when he contemplates the mysteries of eternity, of life, of the marvelous structure of reality. It is enough if one tries merely to comprehend a little of this mystery every day."

Albert Einstein

Knowledge utilisation is the process of making knowledge valuable by making it suitable for social or economic use and for translation into competitive products, services, processes and new commercial activities (definition based on the National Valorisation Committee 2011). Over the past decades, such practical applicability of research has increasingly become a prerequisite for obtaining government funding in the Netherlands and elsewhere. Yet, when asked why they spend their unpaid evenings and weekends performing experiments, analysing data and writing papers, few fundamental researchers will reply: "Because I am trying to save the world." or "Because my research will give a real boost to the Dutch economy." I, for one, might reply: "Because cells produce extracellular vesicles, and I really want to understand why."

I believe that the intrinsic curiosity that drives many fundamental researchers is what allowed mankind to develop into the modern society that we are. An example of a purely curiosity-driven finding is the discovery of the hyperthermophilic bacterium *Thermus aquaticus* that lives in hot springs in the Yellowstone National Park [1]. While this beautiful finding showed that life is possible under circumstances (i.e. at temperatures) that were previously thought incompatible with life, no one at the time would have anticipated that the thermoresistant DNA polymerase of *Thermus aquaticus* would form the basis for one of the most important biotechnological inventions of the late 20th century, the polymerase chain reaction (PCR). PCR is now one of the most used laboratory techniques in molecular biology research and has substantially improved and accelerated the diagnostic process for several diseases, including viral and bacterial infections.

Obviously, it is unlikely that the research presented in this dissertation, or most other fundamental studies, will have a similar impact on science, healthcare and society as the discovery of *Thermus aquaticus*. However, to quote Thomas Levenson of the Massachusetts Institute of Technology, "Science is a set of ways of

thinking and acting through which, sometimes, results emerge. Failure, "wasted" time, is intrinsic to that process. It's impossible to do science without a tolerance for uncertainty over the years or decades it can take to reveal what many discoveries actually mean." [2] Moreover, "Science as a body of knowledge is powerful, valuable, essential to human well-being. Science as a human process is wasteful, necessarily — and even more, usefully — so." [2] The major and, in my opinion, most relevant societal gain of the studies in this dissertation is that they make a contribution to the body of human knowledge (however little it may be).

Societal and economic relevance

On a less philosophical and more practical note, this thesis describes a number of fundamental studies investigating extracellular vesicles (EVs) as a novel mechanistic link between exposure to respiratory toxicants such as cigarette smoke and the development of respiratory exposure-associated chronic diseases. Despite extensive efforts to control smoking prevalence, smoking currently causes 10% of all deaths worldwide [3, 4]. It is projected to continue making an important contribution to the public health burden in the coming decades, in terms of productivity losses, health expenditures and mortality [3, 5]. Additionally, respiratory exposure to air pollutants, especially in urbanised areas, is also an important public health concern [6]. Smoking and exposure to high levels of air pollution are both associated with increased risks for developing cardiovascular disease, chronic lung disease and cancer [4, 6]. Improved understanding of how respiratory exposures cause these diseases is highly relevant, as it forms the first step for developing novel preventive and therapeutic strategies. In this thesis, we have found that cultured lung cells exposed to cigarette smoke release procoagulant EVs which may contribute to the increased cardiovascular risk in smokers, and which may even be involved in lung pathogenesis. While we did not find any significant changes in circulating procoagulant EVs between young healthy smokers and non-smokers, only a sub-group of smokers may respond to respiratory exposures with elevated concentrations of circulating procoagulant EVs. In that case, these EVs could be used as a non-invasive biomarker of risk to identify those individuals that are susceptible to developing chronic disease in response to respiratory toxicants. We have also shown that the clinically tested antioxidant NAC and its active metabolic product glutathione can prevent the release of procoagulant EVs in cells exposed to smoke. Although these are very early stage *in vitro* findings, they provide a rationale for testing the efficacy of NAC to prevent cardiovascular adverse events in individuals with a history of exposure to respiratory toxicants, such as COPD patients.

Target groups

At the current stage, the findings of this research project are mainly relevant to the scientific community, which may use them as the basis for additional fundamental and clinical studies. As suggested in the general discussion of this dissertation (chapter 9) and in the previous section, future research could focus on determining whether circulating EVs can serve as biomarkers of exposure to respiratory toxicants and/or for the risk of developing disease. For this purpose, sensitive, specific and fast methods for the detection of procoagulant EVs in plasma should be developed. Moreover, it could be tested whether NAC can benefit additional target groups besides the subgroup of COPD patients who currently receive NAC for its mucolytic activity. These intermediate steps are required before the findings described in this thesis can be used by the pharmaceutical industry as a basis to develop novel EV based screening tests or before NAC may be implemented in additional target groups in the clinic.

Products and innovation

An important aspect of the work presented in this dissertation was the development of two techniques; one for reliable detection of EVs and EV subtypes in cell culture media, and one for isolation of EVs from cell culture media. While we are not the first ones to use these techniques, and no commercial benefit arises from publishing our protocols, making the protocols publicly available is a valuable contribution to the scientific community. While EVs attract increasing attention and interest from the biomedical research community, only relatively few large and specialised research groups have the expertise, funding and equipment to perform EV isolation and characterisation at the highest quality level. Unfortunately, applying these highest quality techniques as a standard for daily use in smaller or less specialised laboratories seems almost impossible. To prevent a large number of low quality studies being performed with suboptimal EV isolation techniques, such as commercial precipitation kits, techniques need to be developed that are cost-effective, easy and fast to perform. These techniques should also be reliable in terms of specificity for EV detection and in terms of yield and purity for EV isolation. We think that these prerequisites are met by the bead-based flow cytometry that we describe for EV detection and the ultrafiltration with size exclusion chromatography that we describe for EV isolation. Therefore, the publication of these protocols should be a valuable contribution to the EV research community.

Personal development of the candidate and contributions to the scientific community

This PhD project has been the result of a proposal written by the PhD candidate. During the proposal writing, as well as during the PhD project itself, the candidate learned a lot about planning, performing and writing about scientific research. Furthermore, the candidate has attended several specialised courses and gained teaching experience as a tutor for Bachelor and Master students. The research was performed with a supervision team from two departments at Maastricht University, Medical Microbiology and Respiratory Medicine. Moreover, a lot of the work was done in close collaboration with researchers from the departments of Toxicology and Pharmacology, Toxicogenomics, Human Biology, and Biochemistry. Thereby, this project has brought together experts from different fields of biomedical research and has resulted in an intensive exchange of knowledge and in shared use of specialised equipment. This group effort was largely initiated by the PhD candidate and has resulted in two original research papers in international peer-reviewed journals with the PhD candidate as first author, as well as four publications with the PhD candidate as second author. Two review articles have also been published with the PhD candidate as first author, providing other researchers with an overview of the candidate's field of expertise. Moreover, several additional manuscripts are currently submitted or in preparation for publication, in order to make the findings originating from this PhD project broadly available to the scientific community. Parts of the work presented in this thesis have also been presented as posters and oral presentations at several conferences, including the annual meetings of the International Society for Extracellular Vesicles and of the European Respiratory Society.

Conclusion

The major value of the work presented in this dissertation is its contribution to the scientific body of knowledge. We have initiated novel collaborations between researchers from different departments and different fields of expertise. Moreover, we have published protocols that may be of value to the EV research community and have provided evidence that respiratory exposures induce the release of procoagulant EVs, which may contribute to chronic disease risk. These findings may form the basis for additional fundamental and clinical research and may eventually lead to the development of novel screening tests and/or strategies for disease prevention and management.

References

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