Delivering and phenotyping mouse models for the respiratory community: a report on the Biochemical Society Workshop

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Abstract
The IMPC (International Mouse Phenotyping Consortium) was launched recently, and its aim is to develop and phenotype mouse knockouts of 4000 genes over the next 5 years and, ultimately, of all 20000 or so genes in the mouse genome. As part of the IMPC, the MRC (Medical Research Council) also launched a call for MRC mouse networks, where groups of U.K.-based researchers could form a consortium based around a particular area of research. Members of the respiratory research community formed the RDDRC (Respiratory Development and Disease Research Consortium) to consolidate and develop respiratory phenotyping methods suitable for high-throughput screening. This paper, arising from a Biochemical Society workshop held in London in 2012, highlights the purposes of the RDDRC and the needs of the respiratory research community.

Key words: high-throughput screening, International Mouse Phenotyping Consortium, knockout mouse, Medical Research Council, phenotyping, respiratory system

BACKGROUND AND AIMS

In December 2012, a Biochemical Society workshop entitled ‘Delivering and phenotyping mouse models for the respiratory community’ took place in London. The aims of the workshop were to inform the audience about the purposes of the MRC (Medical Research Council) RDDRC (Respiratory Development and Disease Research Consortium) and discuss the needs of the respiratory research community. The workshop presented the facilities and outputs available through the MRC mouse network and the IMPC (International Mouse Phenotyping Consortium) that will be invaluable over the next 10 years. Information on the current phenotyping platforms relevant to respiratory research were presented from both MRC Harwell and the Wellcome Trust Sanger Institute, as well as expert practical knowledge on state-of-the-art mouse genetic and bioinformatic tools available for researchers. The current needs of the respiratory research community were discussed along with outlining plans for a funding application from the RDDRC to boost mouse lung phenotyping thereby enhancing respiratory research.

BURDEN OF RESPIRATORY DISEASE

Respiratory disease remains a major cause of morbidity and mortality worldwide. Recent data from the WHO (World Health Organization) suggest that, of the top five leading causes of death in humans worldwide, three are due to respiratory disease [COPD (chronic obstructive pulmonary disease), pneumonia and lung cancer] behind cardiovascular disease and stroke [1]. Lung cancer has the highest mortality of any cancer. In the U.K. alone, almost 20% of children and 15% of adults have asthma, yet this was an uncommon disease 70 years ago. Lung failure is the end point for many lung diseases; there is no cure for lung failure apart from organ transplantation. Lung transplantation has a 5-year survival of just 50% and the scarcity of donor organs makes this an unrealistic option for most patients. Effective treatments for many respiratory diseases, particularly degenerative diseases of lung structure such as COPD, are limited and the outlook for large numbers of patients is bleak. It is salutary to reflect that treatment has changed little for these common conditions since the middle of the last century. The economic implications of...
respiratory disease are enormous. According to a British Thoracic Society report, respiratory illnesses cost the NHS more than any other disease area (£2576 million in 2000) [2]. Despite the huge worldwide clinical and economic burden of lung disease, funding for respiratory research remains well below that of comparable disorders. There is therefore a significant need to increase basic understanding of lung biology with a hope that this may identify novel future diagnostic and therapeutic targets. How this is best achieved is a matter of debate but, clearly, a better understanding of the function of genes which determine developmental and physiological networks involved in lung diseases will be extremely important.

THE 21ST CENTURY MOUSE

Although there has been progress in clinical research studying human populations and disease cohorts, model systems have been invaluable in allowing researchers to develop and test hypotheses related to specific research questions in a controlled way. Following pioneering work by Capecchi, Evans and Smithies [3], who were awarded the 2007 Nobel Prize for Medicine and Physiology, the discovery of targeting and manipulation of specific genes in the mouse, the toolkit now available to mouse biologists is extraordinary. This work has revolutionized the study of mammalian biology and allows critical manipulation and validation of the function of genes identified in man from a wide variety of “-omic” studies.

Following on from a number of pilot programmes undertaken internationally in recent years, the IMPC (https://www.mousephenotype.org/) was established in 2011 to capitalize on the genetic and genomic technologies now available [4]. The IMPC itself builds on the IKMC (International Mouse Knockout Consortium; http://www.knockoutmouse.org/), which has been generating the targeted embryonic stem cell lines needed to produce mutant mice. The aim of the IMPC is to systematically phenotype mouse knockouts of every gene in the mouse genome, some 20000 plus genes. The phenotyping information and mutant mice are available to the scientific community [5]. This uniform approach has key advantages; for example, all mice will be made using the same targeting construct and mutant mice will be generated on a single uniform background strain, C57BL6/N. These two key points mean that data from different experiments, undertaken at the same centre, can be directly compared in the knowledge that phenotypes are not due to differences between background strains. This is particularly important in large-scale phenotyping projects, as it is known that mouse strains exhibit considerable and important differences in lung structure [6], airway physiology [7] and response to parenchymal regenerative cues [8].

RDDRC AND IMPC

The current IMPC plan is to phenotype 5000 mutant mouse lines by 2016, scaling up to all 20000 protein-coding genes by 2021, at a number of centres worldwide capable of high-throughput phenotyping. As these centres are located across North America, Australia, Asia and Europe (see http://www.mousephenotype.org), this is truly an international project that will have universal benefit. Each mutant mouse line will go through a broad-based phenotyping pipeline to assess all major organ systems and areas relevant to the major human diseases (Figure 1).

The RDDRC is one of 15 consortia that make up the MRC mouse network (https://mrcmousenetwork.har.mrc.ac.uk). This network was created shortly after the IMPC was established to provide U.K. scientists with a platform to engage with the IMPC. As one of the consortia within the mouse network, the RDDRC aims to: (i) make use of phenotyping data provided by IMPC; and (ii) utilize IMPC mice to generate novel in vivo models of respiratory disease and enhance the existing research programmes of participating principal investigators and the wider respiratory research community. Currently, the RDDRC comprises 28 principal investigators from eight U.K. universities. Each centre has its own particular research expertise and together cover all of the major areas of respiratory research. Across the centres is a strong theme of clinical translation with many investigators, based within NIHR (National Institute for Health Research)-funded specialist Respiratory BRUs (Biomedical Research Units), BRCs (Biomedical Research Centres) or in stand-alone programmes that focus on translational science, first-in-man studies and later-phase clinical trials. The RDDRC is therefore ideally placed to take forward discoveries using IMPC mutants. Following consultation with consortium members, the RDDRC put forward a list of genes that principal investigators wish to obtain mouse mutants for. The listed genes are all part of existing research programmes, as there is such an immediate need for these particular mice.

In many cases the genes requested are embryonic lethal because they are critically required during development. In these cases, groups within the RDDRC can choose to generate conditional knockouts of their genes of interest using relevant Cre lines (see below). The generation of conditional-ready floxed alleles can be undertaken either within individual research centres or in collaboration with MRC Harwell, who co-ordinate the mouse network.

LARGE-SCALE PHENOTYPING FOR RESPIRATORY FUNCTION IN MICE

The primary phenotyping pipeline in IMPC consists of a number of different non-invasive tests undertaken in a systematic way. It is therefore imperative that no one test adversely affects the subsequent tests in the pipeline. This clearly limits respiratory phenotyping, prohibiting invasive lung mechanics and airway sensitization. Within these constraints, the most suitable system for measuring high-throughput respiratory function is perhaps unrestrained whole-body plethysmography. Although this technique has important limitations in disease studies [9], it may have a role in high-throughput screening of mutants. The RDDRC have been working closely with MRC Harwell to pilot two separate tests for respiratory function: these are a hypoxia challenge, which reflects parenchymal function, and a
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Figure 1 The core IMPC phenotyping pipeline

The core adult and embryonic core phenotyping pipeline agreed by participating centres, along with tests that are currently being piloted, are shown. Non-mandatory tests may only be conducted at some of the phenotyping centres. CSD, combined SHIRPA (SmithKline Beecham, MRC Harwell, Imperial College, Royal London Hospital, phenotype assessment) and Dysmorphology. This Figure was reproduced with permission from the International Mouse Phenotyping Consortium website (http://www.mousephenotype.org/impress).

methacholine dose–response curve to measure airway function. The plethysmography protocols established at MRC Harwell contains 16 chambers (Buxco) enabling high-throughput measurement of mouse cohorts, importantly enabling simultaneous recording of respiratory parameters, such as breathing frequency, PenH and tidal volume, necessary for data consistency. Initial results are promising; not only can measurable differences in airway and parenchymal lung function be detected using these tests, but consistent results for the methacholine challenge have been obtained from Baylor College of Medicine, Houston, TX, U.S.A., who are using an identical protocol with that at MRC Harwell (S. Wells, personal communication). At the end of the phenotyping pipeline, samples will be taken, including blood, FACS analysis of spleen performed and tissues removed for histopathology. Importantly, at some centres, including MRC Harwell, lungs will be inflation-fixed prior to embedding enabling optimal examination of lung architecture. Other IMPC phenotyping centres, Toronto Center for Phenogenomics in Canada and Institute Clinique de la Souris in France, are piloting different respiratory phenotyping tests and it is hoped that, once pilots are complete, a standardized respiratory assessment protocol will be adopted by most, if not all, centres.

Embryonic screening

Over the past few years, it has become evident that many of the genes and pathways important for lung function are critically required for lung development [10–12]. It is therefore unsurprising that deletion of many of these genes will not be compatible with post-natal life; in fact, it is estimated that mutations in at least one third of genes in the genome will be lethal during embryogenesis of which 20% will be lethal around birth (peri-natally) (S. Wells personal communication).

For those IMPC lines that are not post-natally viable as homozygotes, or where they are determined to be subviable (with the frequency of surviving homozygotes lower than expected), separate embryonic screening is being carried out. The precise details of embryonic screening, such as the time points examined, varies between phenotyping centres, but the recently established DMDD (Deciphering the Mechanisms of Developmental Disorders) programme aims to identify all embryonic lethal knockout lines generated at the Wellcome Trust Sanger Institute over the next 5 years [13]. At MRC Harwell, embryonic phenotyping currently consists of a viability test, as well as lacZ reporter staining at E12.5 (embryonic day 12.5). Depending on viability at this time point, lines are then triaged for an additional viability test and documentation of morphology by 3D imaging at E9.5 or E14.5–E15.5. The purpose of the embryonic screen is to determine minimal information about where and at what stages the gene of interest is expressed, the approximate stage of death and to note any morphological defects, if present, in a manageable and high-throughput manner.

lacZ screening

Another aspect of the constructs being used to make the IMPC mouse lines is the inclusion of a lacZ reporter gene insertion enabling X-Gal (5-bromo-4-chloroindol-3-yl β-D-galactopyranoside) staining of heterozygous mice to reveal where each gene product is expressed [9]. Figure 2 shows one of the
targeting constructs being used for IMPC (maps of additional constructs used in IMPC can be found at http://www.knockoutmouse.org/about/view-all-ikmc-allele-types). MRC Harwell, the Wellcome Trust Sanger Institute and other international centres currently carry out lacZ screening of adult mice and on E12.5 embryos. During the workshop, results from the Sanger lacZ screen were presented, showing that approximately 30% of mutant lines exhibit lacZ expression within the respiratory system (Figure 3). These data are likely to be a rich source of novel lung genes (lacZ expression patterns can be viewed at http://www.mousephenotype.org).

**AVAILABILITY AND USE OF GENETICALLY ALTERED MICE FOR RESPIRATORY RESEARCH**

One way to circumvent early lethality caused by a particular gene deletion and enable analysis of gene function in the adult is to make use of Cre/lox technology to conditionally delete a gene of interest in a specific tissue or cell type. In respiratory biology, the two major groups of Cre lines utilized are epithelial Cre lines and myeloid Cre lines.

During the workshop, an excellent overview of the genetic tools available for tissue specific manipulation of respiratory genes was presented, reviewed in Rawlins and Perl [14]. The merits of using a Cre deleter line compared with inducible manipulation of a gene, for example the Tet-on system to switch on particular gene transcription or the Tet-off system to switch it off, were discussed. Frequently, the choice of which type of Cre line to use depends on at which stage to switch a gene on or off, i.e. from the beginning of its expression or at a later point in time, for example, in the adult lungs.

In addition, we heard about the challenges of developing mesenchymal- and fibroblast-specific Cre drivers for the lung, an area that is of key importance for future lung research if we are to understand the function of genes within mesenchyme-derived tissues, as well as in the more easily targeted epithelium. This is likely to be particularly important in human diseases such idiopathic pulmonary fibrosis and COPD. Another valuable resource is the EUCOMM tools project (www.knockoutmouse.org/about/eucommtools), which will generate a ‘cre zoo’ of inducible Cre deleter strains available to the research community.

**IN-DEPTH (SECONDARY) PHENOTYPING**

The most important role of the primary phenotyping platform described above is that it provides an initial indication of whether a particular mutant line is of interest for further, more detailed, investigation. Within the RDDR consortium there is established expertise in secondary phenotyping relevant to specific human lung disease, including analysis of alveolar development and regeneration, lung development phenotyping, measurement of invasive airway mechanics, lung injury and fibrosis models, genetics and pharmacogenetics of asthma, tissue slice and cellular models of disease, bacterial and viral infection models, models for studying surfactant biology, and analysis of stem cells. Individual members, or collaborative groups from the consortium, will obtain mutant mice for a particular gene(s) of interest, following primary phenotyping, for detailed secondary phenotyping. Importantly, meetings of RDDRC members, such as this Biochemical Society workshop, have enabled discussions identifying the most appropriate phenotyping tests relevant to a particular lung defect or disease. These meetings have also led to new collaborations being formed between RDDRCC members.
OUTCOMES FROM THE WORKSHOP AND FUTURE ACTIONS

The workshop concluded with discussions about the possibilities for a potential RDDRC funding application. With additional funding, the consortium could build on data obtained from the primary pipeline to take forward lines with respiratory phenotypes and establish detailed secondary phenotyping using the broad expertise available within the RDDRC. It is also likely that data obtained by RDDRC members using IMPC mouse lines will be useful for wider cross-cutting themes, such as immunology or tissue fibrosis. One of the strengths of the mouse network is that there is overlap between different consortia within the network; this has led to novel research projects being undertaken by members of different consortia.

The creation of a respiratory specific network has already proved to be an excellent way to find out about the needs of the respiratory research community and to foster new collaborations. We hope that the RDDRC will continue to grow and provide a strong voice with which to highlight the importance of basic and translational respiratory research both in the U.K. and worldwide. Details of how to join the network can be found at https://mrcmousenetwork.har.mrc.ac.uk.

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