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SERIES "GENETICS OF ASTHMA AND COPD IN THE POSTGENOME ERA"
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Transgenic and gene-targeted mice as models for chronic obstructive pulmonary disease

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ABSTRACT: Animal models play an important role in the understanding of the pathogenesis of chronic obstructive pulmonary disease (COPD). The applicability of findings to human COPD depends upon several factors, including the disease model, and similarities in mouse structure and function between species.

There are many examples in the literature of transgenic mice that have contributed to the understanding of COPD. Several studies demonstrate the complexity of inflammatory networks and how unexpected findings in animal models have led to the search for new potential mediators in human disease.

Gene-targeting studies into α_1-antitrypsin (α_1-AT) and emphysema in mice have demonstrated that the genetic locus for α_1-AT in mice is very complex and that the loss of one gene is lethal in embryo lung development. This underlines the differences between mice and humans that limit the ability to translate between systems in some instances. Gene targeting has also highlighted complex roles for transforming growth factor-β in COPD and has been used to determine important molecules and pathways in COPD.

Both transgenic and gene-targeted models suffer limitations and their applicability to human chronic obstructive pulmonary disease may be dependant on several factors, some of which are still being learnt. The more that is known about similarities and differences, the better the knowledge will be that is gained to develop for chronic obstructive pulmonary disease.

KEYWORDS: α_1-Antitrypsin, chronic obstructive pulmonary disease, collagenase, gene-targeted mice, tumour necrosis factor-β, transgenic mice

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Animal models have played an important role in the understanding of the pathogenesis of chronic obstructive pulmonary disease (COPD). Animal models figured prominently in the origin of the elastase–antielastase hypothesis in the 1960s, when GROSS *et al.* [1] found that instillation of elastases led to emphysema in rodents. With the advent of genetic engineering, the capacity is now available to specifically overexpress and delete individual gene products in mice, allowing for highly controlled experiments in mammals. These techniques provide opportunities to dissect disease pathways *in vivo*. However, the applicability of findings to human COPD depends upon several factors, including the disease model, and similarities in mouse structure and function between species. The present author's hypothesis is that general mechanisms are likely to be well conserved, but important details may differ considerably; hence, care in translation from mouse to humans is required.

TRANSGENIC MICE

To generate transgenic mice, the cDNA of interest is linked to a promoter (and a polyA tail), which

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is often lung-specific, and the DNA or ''transgene'' is microinjected into pronuclei of fertilised eggs to randomly integrate genetic material into the genome [2]. Upon placement of eggs into the oviduct, offspring may then express the transgene. Newer developments allow conditional (cell-specific) and inducible expression. Inducible expression avoids the developmental expression of the transgene. This is particularly important in the study of COPD, to avoid confusion between destruction and enlargement of airspaces that define emphysema, and enlarged airspaces due to failed septation impairing alveogenesis during lung development (fig. 1). Lineage-specific promoters (or knock-in technology) linked to colour markers that tag particular groups of cells can also be used. These techniques will become very useful in the identification of specific stem cells in the future.

Transgenic mice have been used in COPD to overexpress a particular gene product to obtain a COPD phenotype, usually airspace enlargement. Hence, this technique will allow the deduction that ''if'' this particular gene is expressed at high levels, it ''could'' lead to aspects of COPD. If it is known that the protein is in fact elevated in COPD, this provides strong evidence that it contributes to pathology. There are now many examples in the literature of transgenic mice that have contributed to the understanding of COPD.

One of the earliest applications of transgenic technology to COPD was the overexpression of collagenase-1 (matrix metalloproteinase (MMP)-1) resulting in airspace enlargement [3]. This challenged the elastase–antielastase hypothesis, thereby placing collagen squarely in the picture. A limitation of the study [3] is that while expression was lung specific in some lines (despite being driven by the haptoglobin promoter), it was not inducible. However, the authors did not detect expression of collagenase in some lines during early development. The role of collagen in COPD is complex. Clearly, destruction of an alveolar unit requires loss of all cells and extracellular matrix including collagen; however, total collagen content in COPD is actually increased, with submucosal airway fibrosis likely to contribute to airflow obstruction. Polymorphisms in the collagenase promoter that cause higher levels of collagenase expression actually correlated with protection against decline in forced expiratory volume in one second in the large lung health study population [4]. However, mice do not possess MMP-1 but do express the two other MMP collagenases (MMP-8, neutrophil collagenase or collagenase-2, and MMP-13 collagenase-3), which compensate for lack of collagenase-1 in rodents.

Overexpression of interleukin (IL)-13 [5] and interferon (IFN)-γ [6] represent two important examples of inducible, conditional transgenes leading to emphysema in adult mice. Overexpression of the T-helper cell (Th)2 cytokine IL-13 resulted in inflammation and lung destruction that was metallo- (MMP-9 and MMP-12) and cysteine proteinase-dependent. These mice also exhibit airway remodelling with goblet cell hypertrophy and subepithelial collagen deposition. MMP-9-mediated transforming growth factor (TGF)-β activation was responsible for collagen remodelling in this model [7]. Whether IL-13 is overexpressed in human COPD is currently under investigation. This mouse also supports the ''Dutch hypothesis'', which states that asthma and COPD have common underlying mechanisms. Overexpression of the Th1 cytokine IFN-γ also resulted in inflammation and proteinase-dependent emphysema. Compared with the IL-13 transgenic mouse, the inflammatory component with IFN-γ was more subtle, apoptosis was prominent, and there was no associated airway pathology. These are just two examples that demonstrate the complexity of inflammatory networks and how unexpected findings in animal models have led to the search for new potential mediators in human disease.

Gene-targeted mice

Prior to the advent of gene targeting, several natural mutant mice were known to develop airspace enlargement, including tight skin (Tsk+/-) [8], pallid [9], blotchy [10, 11] and beige mice [12]. The genetic defects for these mice have subsequently been uncovered. Tsk mice have a mutation in fibrillin-1, a matrix protein that is an important component of elastic fibres [13]. While the consequences of this mutation on lung development and repair are clear, the relationship between the genetic defects in the other natural mutant mice to lung

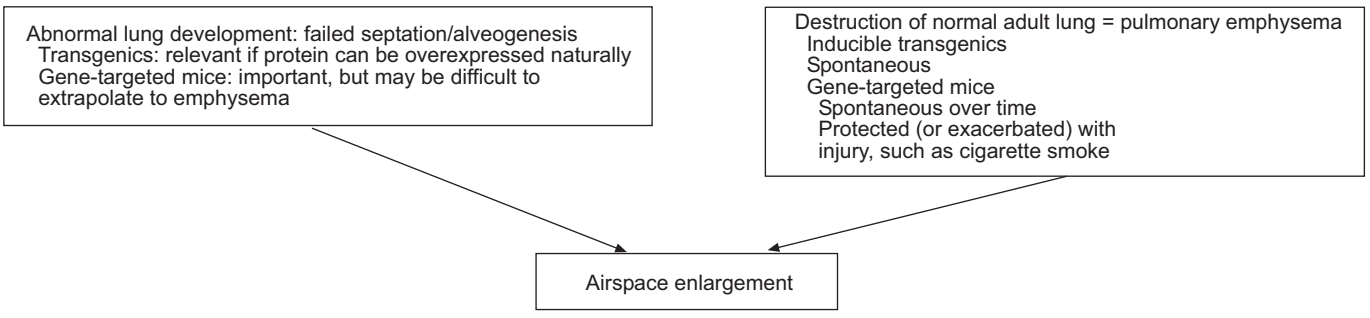

FIGURE 1. Factors resulting in airspace enlargement in mice. Mice may obtain enlarged airspaces due to abnormal development or destruction of mature alveoli. Failed septation during alveogenesis occurring in transgenic mice might have biological importance if the overexpressed protein has the capacity to be overexpressed in human lung development. Abnormal alveogenesis in knockout mice suggests that the product is made and if, for some reason, lost, this could result in disease. Neither mechanism is directly relevant to pulmonary emphysema, which is defined by destruction of airspaces that were previously normal. This phenotype may be observed in mice *via* one of the following: by inducing a transgene during adulthood, spontaneous occurrence over time in gene-targeted mice, and by administering a relevant injurious agent (*e.g.* cigarette smoke) to gene-targeted mice. The mice can then be compared with wildtype mice to determine a role in chronic obstructive pulmonary disease.

structure is not yet apparent. Emphysema in some of these strains, such as the pallid mouse [14], has been attributed to reduced α_1-antitrypsin (α_1-AT) levels; however, if similar to humans, the levels of α_1-AT are not low enough to cause emphysema. In fact, the genetic locus for α_1-AT in the mouse is very complex, with four to five genes (depending upon the strain) that encode proteins >95% homologous to each other [15], two of which inhibit neutrophil elastase [16]. Knocking out one of these genes (α_1-AT no. 2), which only inhibits one-third of neutrophil elastase activity, is lethal in the embryo prior to lung development (unpublished data). This highlights differences between mice and humans that limit the ability to translate between systems in some instances.

Loss of function by gene targeting during foetal development may also result in abnormal lung development and consequent airspace enlargement. While this is not pulmonary emphysema *per se*, the lack of a critical constituent does suggest that the protein is necessary for development. There are now many examples of gene-targeted mice that undergo abnormal alveogenesis, including mice with loss of transcription and growth factors. For example, members of the large family of fibroblast growth factors (FGF) are essential for several stages of mammalian lung development. Lungs of gene-targeted mice lacking receptors for both FGF receptor-3 and -4 (but not single knockouts) have markedly impaired alveogenesis with increased synthesis of collagen [17]. Platelet-derived growth factor-A null mice lack myofibroblasts, which are a key source of tropoelastin and are required for alveolar septation [18, 19]. Mice deficient in extracellular matrix proteins, particularly those involved in elastic fibre formation, develop airspace enlargement [20]. Elastin -/- mice die within 48 h of birth, which precedes alveolarisation, but abnormal lung development can be appreciated then and in heterozygous mutant mice [21]. Loss of microfibrillar proteins, such as latent TGF-β binding protein-3 [22], -4 [23], fibulin-5 [24], and fibrillin-1 [20], all also have abnormal lung development.

The Tsk +/- mice previously described have markedly abnormal lung development [25]. The abnormality of lung structure secondary to loss of fibrillin-1 allows sequestration of TGF-β within the matrix and this appears to lead to abnormal alveolar septation [25]. Interestingly, administration of TGF-β-neutralising antibody to these mice postnatally produced an increase in alveolar septation.

Use of gene-targeted mice has also demonstrated complex roles for TGF-β in COPD. TGF-β -/- mice die of overwhelming inflammation within 1 month of birth, limiting their utility to study COPD. However, mice deficient in the β_6-integrin, lacking avb6, fail to activate latent TGF-β within the lung. These mice also develop macrophage-rich inflammation with excess MMP-12 production. Avb6 -/- mice undergo normal alveolar development but, over time, they develop spontaneous emphysema [26]. It is known that TGF-β inhibits MMP-12 production. Back-crossing avb6 -/- to MMP-12 -/- mice abrogates emphysema, as does crossing these mice to transgenic mice overexpressing TGF-β. Although total absence of TGF-β releases the brakes on inflammation, too much TGF-β leads to airway fibrosis, another important component of COPD. The β_6-deficient mouse represents an example of a gene-targeted mouse with normal development but spontaneous emphysema with ageing. This represents another use of gene-targeted mice to determine important molecules and pathways in COPD.

Limitations of transgenic and gene-targeted mice

Transgenic technology depends upon random introduction of the gene of interest into the recipient genome. This may interfere with the function of other genes, and thus obtaining identical phenotypes in multiple founders is important to assure that the phenotype is related to the transgene itself and is not an integration effect. Gene targeting is based upon homologous recombination of the mutation within the genetic locus of the gene of interest and does not suffer from this limitation.

As discussed, loss of a gene with a resultant phenotype implies that it plays an important functional role, particularly if applied to a disease model. Introduction of a transgene resulting in a phenotype suggests that it could mediate this phenotype if overexpressed in the disease but is not necessarily relevant. For example, with respect to lung development, expression of a transgene during alveogenesis may lead to developmental emphysema impairing interpretation of its effect on pulmonary emphysema (destruction of mature alveoli). Gene targeting might also lead to abnormal development, the difference here being that developmental airspace enlargement with gene targeting suggests a true developmental role for this gene, whereas artificial overexpression of a transgene does not.

Mice in any given inbred background are genetically identical; however, when mice are not on a pure background, phenotypic differences may be related to background strain differences rather than the genetic manipulation itself. Littermates in the mixed background help but, depending upon the phenotype, might not suffice.

The greatest concern, of course, is the utility of mouse studies in predicting human pathophysiology and pathogenesis. The mouse lung has the same general structure and physiological mechanisms as the human lung; however, there are notable exceptions that make translation to humans difficult. For example, the mouse airway has few submucosal glands and only six to eight branches until the terminal bronchiole is reached, which goes directly to the alveolar duct. Humans have >20 branches before becoming the respiratory bronchiole, a structure not present in mice, which is the site of initial inflammation and genesis of centriacinar emphysema. In addition, mice do not always express proteins identical to those in humans, as seen in the previous example of MMP-1. However, while structure and participation in function may differ, the present author maintains that the general pathological pathways in response to stress (for example, cigarette smoke) are conserved; hence, a unique understanding of human pathobiology can be gained *via* manipulation of the mouse genome. The precision of the translation will depend on a number of factors, some of which are still being learnt about now. The more that is known about both similarities and differences, the better the knowledge will be that is gained from genetic engineering in mice to develop therapy for chronic obstructive pulmonary disease.

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