Medical research

Mucus secretion blocked at its source in the lungs

Irina Gitlin & John V. Fahy

Higher than normal secretion of mucin, a molecular component of mucus, is a feature of many lung diseases. The development of a peptide that blocks mucin secretion in airway epithelial cells might lead to therapies. **See p.949**

Excessive production and secretion of mucins (the gel-forming protein components of mucus) contribute to the formation of mucus that causes blockade of the airways in many lung conditions[1]. Mucins are produced by goblet cells (a type of epithelial cell that lines the airways) and by mucous cells in submucosal glands. They are packaged intracellularly in vesicles called secretory granules. Molecules that stimulate mucin secretion (termed mucin secretagogues), such as ATP, initiate a signalling cascade that results in calcium-ion-triggered fusion of the granule and cell membranes[2]. The proteins that mediate this fusion include SNARE proteins, which are located mainly in the cell membrane, and a calcium-sensor protein called synaptotagmin-2 that is present in the granule membrane. Lai *et al.*[3] report on page 949 that an engineered peptide disrupts the interaction of the SNARE complex with synaptotagmin-2, thereby blocking mucin secretion both *in vitro* and *in vivo*.

The mechanisms underlying disease-associated (pathological) mucus formation in lung conditions such as asthma, chronic obstructive pulmonary disease (COPD) and cystic fibrosis are complex. They include mucin overproduction and hypersecretion, mucus dehydration, oxidative-stress-driven mucin crosslinking, and overabundance of non-mucin polymers such as DNA and actin in mucus[4–6]. The drugs currently approved to tackle mucus problems are designed to improve mucus hydration or to cleave mucin or DNA polymers in mucus. These treatments were developed for cystic fibrosis, and clinical trials show that they improve lung health[7].

However, the mechanisms underlying pathological mucus formation in cystic fibrosis differ from those in other lung diseases, and these mechanistic differences explain divergences in the treatment response to mucus-targeting drugs. For example, DNA concentrations are high in the mucus of individuals with cystic fibrosis but relatively low in people with other types of lung disease[8]. Treatments that cleave DNA are therefore recommended for cystic fibrosis but not for other lung conditions. Similarly, an ion channel called CFTR, which controls mucus hydration by regulating chloride- and sodium-ion transport in airway epithelial cells, malfunctions as a result of the genetic mutations that underlie cystic fibrosis, and CFTR modulator therapy is thus restricted to people with that disease[9]. So there is a major shortage of drugs that target mucus in lung diseases other than cystic fibrosis.

One way to reduce excess mucus in the lungs is to decrease its production. The protein interleukin-13 (IL-13) has a key role in mucin overproduction in asthma, and proteins that bind to the ErbB family of receptors (such as epidermal growth factor, transforming growth factor-α and amphiregulin) contribute to mucin overproduction[10] in COPD. Inhibiting such proteins therefore offers a rational approach to treating mucin overproduction. It is possible that IL-13 inhibitors, already approved for use in treating asthma, might decrease airway epithelial mucin stores, but clinical trials of these inhibitors did not include measurements of mucin stores in airway epithelial cells (see, for example, ref. 11). One clinical trial[12] of an inhaled inhibitor of epidermal growth factor receptor did not find statistically significant decreases in mucin stores of airway epithelial cells in people with COPD.

An alternative strategy for treating excess mucus is to disrupt the fusion of mucin storage granules with the cell membrane (Fig. 1), thereby blocking secretion. Mucin secretagogues activate cellular receptors and generate an intracellular calcium-ion signal to initiate fusion. Because the calcium-regulated protein complexes that drive membrane fusion and mucin secretion are well understood, it should be possible to design inhibitors of this process, and so provide a new and broadly applicable approach to treating mucin hypersecretion in asthma, COPD and other lung diseases. However, mucin secretion is an essential normal response in airways and must be primed to respond to myriad inhaled disease-causing agents and toxins. Whether drugs that block mucin secretion could be given in doses that would prevent mucus-associated disease but not impair a protective mucus response is unknown and requires further investigation.

Lai and colleagues designed a peptide (called SP9) to block the fusion of mucin granules with the cell membrane in airway epithelial

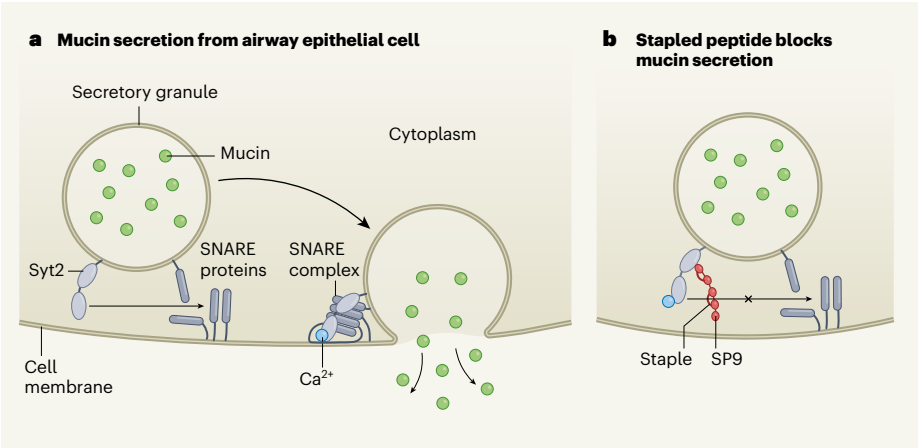

Figure 1 | An approach to tackling harmfully high levels of mucus. In many lung diseases, the airways become clogged with mucus, but few treatments exists to combat this problem. **a**, Mucin, a key constituent of mucus, enters the airways when mucin-containing bodies known as secretory granules fuse with the membrane of airway epithelial cells. This fusion event depends on the interaction between the protein synaptotagmin-2 (Syt2), bound to calcium ions, and SNARE proteins, which rearrange to form what is termed the SNARE complex. For simplicity, only some proteins involved in the fusion event are shown. **b**, Lai *et al.*[3] report a way to block mucin secretion *in vitro* and *in vivo* in mice. Their approach relies on SP9, a peptide containing hydrocarbon 'staples' that stabilize its structure. SP9 binds to Syt2, thereby preventing its binding to SNARE proteins, inhibiting fusion of the secretory granule with the cell membrane and blocking mucin secretion.

cells. SP9 is what is termed a stapled peptide — one that is constrained by a synthetic hydrocarbon 'brace' that stabilizes its structure and locks it into a specific conformation[13]. Stapled peptides have better target affinity and increased cell penetration compared with peptides lacking such staples, and they are less susceptible to being degraded. They are particularly well suited for the disruption of intracellular protein–protein interactions[14], such as those between synaptotagmin-2 in the secretory granule and proteins of the SNARE complex in the cell membrane.

In designing SP9, the authors used knowledge of key amino-acid residues in the principal SNARE-protein-binding partner for synaptotagmins to generate a peptide that inhibits calcium-ion-triggered granule fusion. SP9 also has a cell-penetrating peptide tail to boost its entry into cells. Lai and colleagues' work convincingly demonstrates that the engineered SP9 enters airway epithelial cells to block ATP-stimulated mucin secretion in IL-13-primed airway epithelial cells *in vitro* and *in vivo* in mice.

It remains to be seen whether stapled peptides could be administered by aerosol in sufficient doses and with sufficient safety (especially in a setting of routine administration in chronic illness) to become approved therapeutics for mucus-associated lung disease. But by confirming that it is possible to block calcium-regulated mucin secretion, Lai and colleagues have shown the potential of such an approach as a new therapeutic strategy for lung illnesses associated with mucus pathology, including diseases such as asthma and COPD, for which there is a large unmet medical need.

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Forum: Genetics

Constrained human genes under scrutiny

A higher number of damaging variations in certain genes is associated with an increased likelihood that a man will be childless. A geneticist and an anthropologist discuss what can — and can't — be learnt from this finding. **See p.858**

The paper in brief

- **Some genes are constrained, which means that damaging variants of them are removed from the population by natural selection.**
- **On page 858, Gardner *et al.*[1] investigated the processes underlying this evolutionary process in humans.**
- **They report that having a high overall amount of damaging genetic variation in constrained genes is associated with childlessness in men.**
- **The association is linked to only 1% of the chance of childlessness between individuals, but to larger effects over many generations in a population.**
- **The findings are consistent with the hypothesis that having a greater burden of damaging genetic variation might affect a man's ability to find a mating partner.**

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An evolving understanding of gene constraint

Loss-of-function (LoF) mutations inactivate genes completely. Some genes in a human population are able to 'tolerate' LoF mutations, whereas others, known as constrained genes, cannot — LoF variants in constrained genes tend to be lost over time through natural selection. As a result, fewer people would be expected to have LoF variants in a constrained gene than in an LoF-tolerant gene. A large study of human genetic variation carried out by Lek *et al.* in 2016 identified about 3,000 LoF-intolerant genes[2]. Gardner and colleagues' work might help us to understand how natural selection has constrained them.

It is thought that LoF mutations might affect reproductive fitness — that is, the number of offspring an individual produces. For example, these mutations might reduce the chance of a person living to reproductive age, cause infertility or affect a person's ability to find a mate. About one-third of the 3,000 constrained genes identified in Lek and colleagues' study have been linked to disorders associated with mortality before the individual reaches reproductive age and with reduced fertility (according to the Online Mendelian Inheritance in Man database; https://omim.org). But whether and how the other genes might affect reproductive fitness has been unclear.

To address this issue, Gardner and colleagues analysed rare protein-truncating mutations in the 3,000 genes in more than 300,000 unrelated individuals who are part of the UK Biobank — a database of genetic and health-related information for 500,000 volunteers in the United Kingdom. This cohort is made up largely of individuals between 39 and 73 years old, ensuring that most have, in principle, had the opportunity to reproduce. The authors quantified the overall association of all protein-truncating mutations and gene deletions with reproductive success. Their main finding is that, cumulatively, LoF variants in these 3,000 genes are associated with childlessness in men but not in women. Interestingly, this association is not