PERSPECTIVES

When the lung is stretched, could it be thrombospondin via TGFβ1 peptide activation?

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The lung lumen is fluid filled *in utero*. Chloride channels drive secretion of fetal lung liquid which effluxes from the larynx. Laryngeal tone acts as a pinchcock valve, imparting an intraluminal pressure of 1.5 cmH_2O. Obstruction of fetal tracheal fluid outflow increases intraluminal pressure by about 2- to 3-fold, which results in a marked but quite dysplastic increase in lung size and cellularity. Conversely, chronic drainage of fetal lung liquid causes marked lung hypoplasia. The molecular mechanisms mediating the response of the fetal lung to changes in intraluminal hydraulic pressure are not well understood. Sozo *et al.* (2006) showed that thrombospondin-1 (Tsp-1) expression is the most strongly up-regulated among a large panel of genes in response to artificial obstruction of tracheal fluid flow in the fetal lamb. In an article in the current issue of *The Journal of Physiology*, they have now come up with some temporo-spatial details on this result (Sozo *et al.* 2007).

Tsp-1 is expressed at both the gene and protein level within type 2 pneumocytes and fibroblasts in the periphery of the lung, and its expression is increased at least 3.5-fold in response to obstruction of tracheal fluid outflow, while conversely Tsp-1 is decreased to 35% following drainage of fetal lung liquid. They speculate that Tsp-1 may regulate fetal lung growth in response to mechanical stretch and that its mRNA expression may be regulated transcriptionally by Egr1, a transcriptional factor that is also induced by distension of the fetal lung. However, Tsp-1 activity *per se* was not addressed.

The thrombospondins comprise a family of extracellular glycoproteins that coordinate cell–matrix interaction in diverse systems such as the vasculature, eye and liver by several mechanisms, including stimulation of cell proliferation. One of the better understood functions of Tsp-1 is the localized activation of latent TGFβ family peptides (Crawford *et al.* 1998; Ribiero *et al.* 1999). Latent TGFβ peptides are anchored within the extracellular matrix in complex with fibrillin, which itself has important functions in the correct cross-linking of matrix elastin fibres, as well as playing an essential role in the activation of latent TGFβ complexes (Kaartinen & Warburton, 2003). Recently it has been shown that integrins $\alpha v\beta 6$ and $\alpha v\beta 8$ also interact with an RGD sequence within the latent TGFβ peptide itself and that this interaction is likewise necessary for the liberation of active TGFβ from the latent TGFβ peptide (Yang *et al.* 2007). Since Tsp-1 is also found within this supramolecular TGFβ activation complex and its expression is induced by mechanical stretch, not only in the lung but also interestingly in the lamina cribrosa of the eye (Kirwan *et al.* 2005), it is tempting to speculate that when a tissue is stretched, localized induction of Tsp-1 expression could mediate increased Tsp-1 activity. This could in turn effect localized activation of latent TGFβ1 peptide within the matrix surrounding individual cells in tissues that have been stretched. Running somewhat counter to this enticing mechanistic scenario, Wallace *et al.* (2006) actually found a decrease in whole tissue TGFβ activity in the same fetal lamb lung distension model. Nevertheless, the current study does show that Tsp-1 expression is induced severalfold in a physiologically relevant part of the peripheral fetal lamb lung.

Tsp-1 null mice are available so that it would be interesting to know whether these mice can respond to pulmonary stretch (Lawler *et al.* 1998). Genetic interference with key component parts of the supra-molecular latent TGFβ1 peptide complex produce somewhat disparate effects in the lung. Mutation of fibrillin leads to a Marfan's syndrome phenotype complete with early onset apical bullae in the lung (Neptune *et al.* 2003). More strikingly, mutation of the RGD binding domain within the latent TGFβ1 peptide completely abrogates its ability to bind integrins, whose binding is required for activation of the latent peptide and hence this point mutation in RGD replicates fully null mutation of TGFβ1 (Yang *et al.* 2007). In contrast to fibrillin-1 deficiency, TGFβ1 null mutation does not cause an obvious neonatal lung phenotype. However, when TGFβ1 null mice are raised under standard non-sterile conditions, they develop an aggressive pneumonia that kills them around a month of age (Kulkarni *et al.* 1993). The pulmonary phenotype of Tsp-1 null mice also comprises lethal pneumonia, which is tellingly almost identical to the TGFβ1 null phenotype (Crawford *et al.* 1998). The Tsp-1 null phenotype can be partially rescued with a soluble peptide derived from Tsp-1 that can activate latent TGFβ1. Tsp-1 therefore appears to be an essential component of the TGFβ1 activation pathway.

Tsp-1 has long been appreciated as a major activator of latent TGFβ peptide activity and fibrosis in the rat kidney, whereas inhibition of Tsp-1 ameliorates renal fibrosis (Daniel *et al.* 2003, 2004). It would be interesting to know whether Tsp-1 also plays a targetable role in pulmonary barotrauma, broncho-pulmonary dysplasia, lung fibrosis, COPD and/or asthma, all lung disease entities in which the pulmonary stretch response is thought to be involved.

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