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Human Surfactant Proteins (SP-) A1 and SP-A2: to Include or Not in Surfactant Replacement Therapy, and If Yes, Both or Which One?

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Short Communication

Based on a large number of animal studies, surfactant protein A plays important roles in lung innate immunity under basal conditions and in response to various insults such as infection and oxidative stress. SP-A interacts with the Alveolar Macrophage (AM), the sentinel cell of innate immunity, and regulates many of its functions. These include the ability of the AM to produce cytokines and to carry out phagocytosis of various pathogens. Moreover, the AM proteomic expression profile has been shown to be significantly affected by SP-A. In a study where SP-A -/- mice were treated (or rescued) in vivo with a single dose of human SP-A, the treatment nearly restored the AM proteomic expression to that of the wild type [1], supporting a role of SP-A on AM protein expression. Among the protein groups affected were actin-related/cytoskeletal proteins, which was the major group, proteins regulating inflammatory processes, and proteins related to Nrf2-mediated oxidative stress. Of interest, earlier studies have also indicated a role for SP-A in actin filament dynamics via its ability to enhance cell migration and AM chemotaxis [2], phagocytosis [3], other actin-dependent processes [4-6] and F-actin assembly [7]. The actin cytoskeleton is highly regulated and dynamic, with globular (G) and filamentous (F) actin, under the influence of many actin binding proteins, constantly changing by polymerization, depolymerization, branching, and remodeling, modulating inflammatory response and oxidative stress (Figure 1).

As shown in Figure, signaling via NF-kB [8] and Nrf2 [9] can both depend on cytoskeletal changes, indicating that SP-A-mediated modulation of the actin cytoskeleton could have significant effects on AM responses. Moreover, the SP-A -/- rescue experiment [1] points to the possibility and feasibility of using SP-A as a therapeutic intervention in lung diseases influenced by innate immunity. Furthermore, based on animal studies [10] where small amounts of SP-A elicited significant differences in the AM proteome, it is possible that the SP-A amount needed may not be high, although, further experimentation will be needed to determine optimal amount and efficacy in the clinical setting.

However, humans, (unlike rodents) have two genes, SFTPA1 (SP-A1) and SFTPA2 (SP-A2) with several genetic variants identified for each SP-A1 and SP-A2 gene. These variants have been associated with many lung diseases including neonatal diseases, such as Respiratory Distress Syndrome (RDS) and Bronchopulmonary Dysplasia (BPD) [11]. The SP-A1 and SP-A2 gene products have been shown to differ in several functions related to innate immunity [12] and in surfactant-related functions [13,14]. These differences have been reviewed previously [12] and include differences in their oligomerization status [13,15,16], their ability to form phospholipid monolayers [17], their ability to bind sugars [18], and their ability to enhance the phagocytic activity and cytokine production by the alveolar macrophages and macrophage-like cell lines, respectively [3,19-22], as well inhibit surfactant secretion by Type II cells [13]. In most of these cases SP-A2 appeared to exhibit higher functional activity. However, in preliminary studies [23], we observed that SP-A1 produced a higher reduction in surface tension than SP-A2, with no significant differences observed between SP-A1 and SP-A that contained both SP-A1 and SP-A2. Furthermore, in the presence of oxidative stress the activity of SP-A2 to enhance phagocytosis may be reduced more than that of SP-A1 [3], and the SP-A2 resistance to trypsin is lower than that observed in SP-A1 and SP-A1/SP-A2 [15]. Collectively, these observations indicate that SP-A1 and SP-A2 may each be better for different functions in the lung i.e. either for surfactant-related functions and/or innate immunity. Thus, the addition of SP-A1 and/or SP-A2 in surfactant replacement therapies may be advantageous to both groups of functions, to innate immunity and surfactant, and in some cases and under certain conditions one may be better than the other.

SP-A humanized Transgenic (hTG) mice, generated on the SP-A -/- background where each hTG mouse carries a different human SP-A variant provide models to study the in vivo functional activity of each variant. With these mice we observed differences in the oligomerization pattern of SP-A1 and SP-A2 confirming in vitro findings and also observed that both SP-A1 and SP-A2 may be needed for an extracellular form of surfactant [14]. In addition, we have shown differences in the proteomic profile of AM derived from SP-A1 and SP-A2 hTG mice [24]. In other words, AM constitutively exposed to different human SP-A variants in vivo exhibit different expression profiles, and presumably this reflects different stages of functional activity either in one or more AM functions. In fact, the protein differences observed span several functional protein groups. Among these there were actin-related and cytoskeletal proteins, which was the major group of differentially regulated proteins by SP-A1 and SP-A2, as well as proteins involved in the regulation of inflammation, and proteins related to the Nrf2-mediated oxidative stress response [24]. As shown in Figure, remodeling of the actin cytoskeleton is central to several AM functions. Therefore, SP-A1 and SP-A2 are likely to differentially regulate several AM functions.

In the prematurely born infant, apart from potential exposure of the lung to varied levels of oxidative stress, one of the major complications is infection. Although innate immunity and the surfactant proteins could play a significant role in this process, none of the surfactant replacement therapies include the surfactant proteins SP-A1 and/or

SP-A2, or SP-D [25]. Of interest, surfactant treatment of babies with RDS defined as "simple" or with pneumonia indicated that in the latter group, surfactant treatment was less effective [26]. In addition, after surfactant therapy, neonates with respiratory failure and Group B streptococcal infection, although most of them showed an improvement in gas-exchange, this improvement was slower compared to RDS [27]. Whether the outcome in RDS with or without infection, or in other diseases beyond RDS [28] would or could be better if SP-A is included in the surfactant preparations or given alone remains to be determined. Of interest, the efficacy of surfactant in its current form on bacterial pneumonia in preterm infants, remains an open question. An attempt to assess randomized clinical trials for efficacy of surfactant in late term and preterm infants with bacteria pneumonia could not reach any conclusions one way or another at the present time [29]. However, based on the available literature, and the role of human SP-A1 and SP-A2 on AM functions, it is imperative that considerations for including the surfactant proteins in existing surfactant preparations or using them individually in the absence of surfactant be investigated. Moreover, as noted above, SP-A1 and SP-A2 may also contribute to surfactant-related functions and therefore its potential therapeutic use may benefit these functions as well. Furthermore, considerations as to whether both or which genetic variant(s) for a given condition be used must not be overlooked. These questions must be investigated with further experimentation.

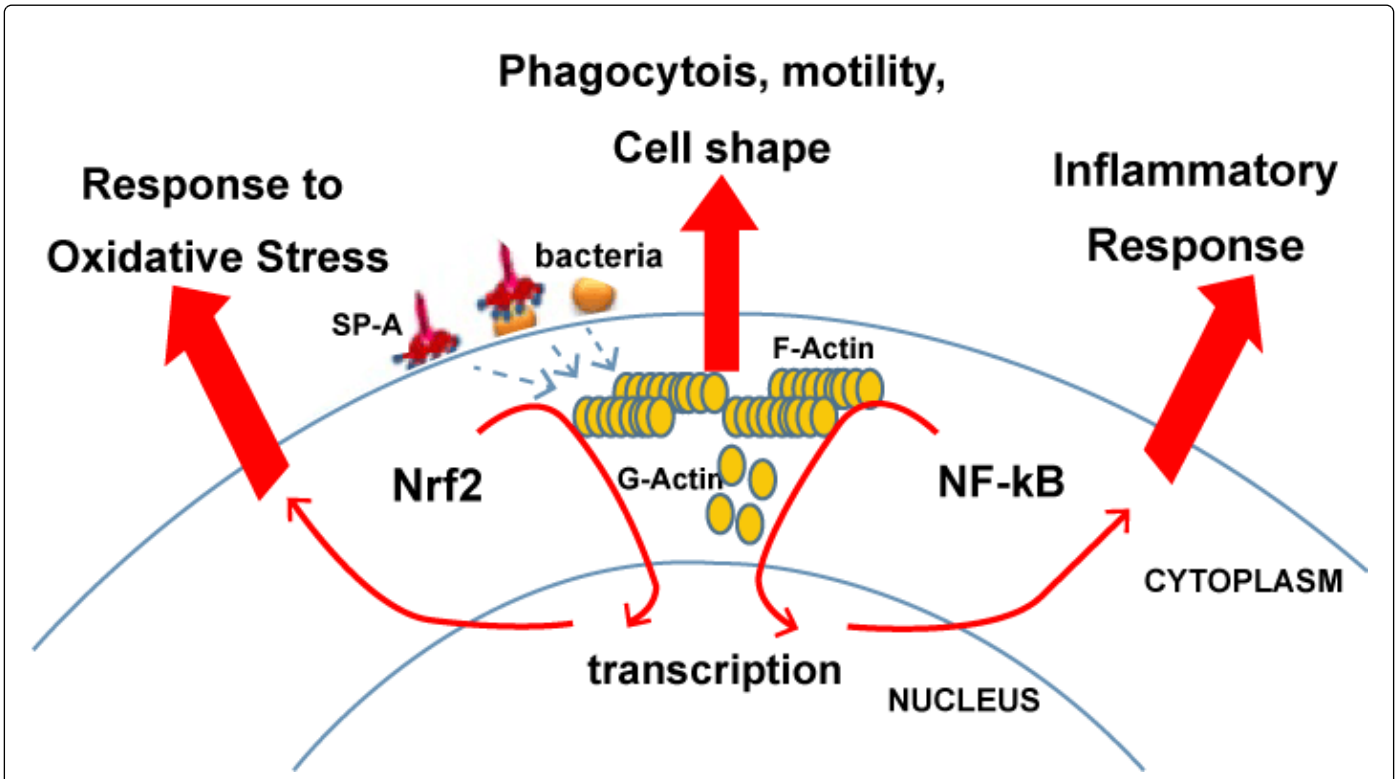

Figure 1: A diagrammatic presentation of potential cytoskeletal/actin-mediated processes in the alveolar macrophage [AM] in response to SP-A; the central role of cytoskeletal changes in the modulation of signaling pathways and the function of AM are shown. Our published studies [1] show that SP-A has significant impact on the AM expression profile and specifically on the cytoskeletal/actin-related proteins. Based on the literature cytoskeletal changes may affect several signaling processes including NF-kB [8] and Nrf2 [9]. These processes in turn via transcriptional regulation modulate the inflammatory response and the response to oxidative stress. In addition cytoskeletal changes are key in AM activities such as phagocytosis, motility, and cell shape. Thus, SP-A, via its ability to alter expression of cytoskeletal/actin-related proteins in the AM, may affect several of the AM functions or responses [i.e. inflammation, oxidative stress, phagocytosis, and other]. For example, human SP-A variants have been shown to differentially enhance the phagocytic activity of AM [3] and mice that lacked SP-A exhibited a lower phagocytic activity and survival [10].

Because low levels of SP-A have been associated with neonatal disease (RDS, BPD) risk, as well as with risk in adult disease (IPF) or condition (lung trauma) [11], enhancing the expression of SP-A1, SP-A2, or both depending on the particular functional need may be another way to increase their levels in neonatal life. Although individuals with lung disease have been shown to have altered levels of SP-A, it is not known whether these reflect change in both gene products, or just one, and if the latter, which one. Given the functional differences between the two gene products and eventually functional differences among the variants of each gene, it is important to have a good understanding of the SP-A1 and SP-A2 gene-specific regulatory mechanisms in order to guide therapeutic decision making. Although differences in the regulation between SP-A1 and SP-A2 have been observed in organ cultures and in cell cultures, the details of the mechanisms are not fully understood [12]. Recent studies have implicated regulatory protein factors [30] and miRNAs [31] in the differential regulation of SP-A1 and SP-A2 expression. For example, some isoforms of the 14-3-3 family of proteins are specific for the translation of SP-A2, but not SP-A1 [30]. Whether these or other factors can in the future be used therapeutically to differentially

regulate specific SP-A1 and SP-A2 expression remains to be determined.

In summary, serious consideration should be given to the question of whether SP-A1 and SP-A2, as well as SP-D (not discussed here in detail), should be included in surfactant replacement preparations or be given as an individual treatment. The mouse rescue experiment [1] discussed above for SP-A indicates feasibility and potentially a functional benefit to the AM as shown by changes in the cytoskeletal/actin-related proteins. The cytoskeletal changes, as depicted in Figure, are central to several AM functions. Innate immunity, being the first line of defense, provides a logical target for therapy, because deranged innate immunity most likely leads to dire downstream consequences. Thus, ensuring a well-functioning AM is essential to lung health. SP-A1 and SP-A2 appear to be important regulators of AM protein expression and function, and using these therapeutically may help ameliorate disease severity in several conditions. Moreover, preliminary evidence indicates that SP-A1 and SP-A2 treatment may have a positive impact beyond innate immunity by benefiting surfactant-related functions, and perhaps clinical cases beyond RDS. Alternatively, understanding the regulatory mechanisms of SP-A1 and SP-A2 could help modulate SP-A1 and SP-A2 levels and therefore this line of investigation should be a research priority. In specific, such knowledge can help identify points of therapeutic intervention to enhance individual expression of SP-A1 and/or SP-A2 under certain conditions.

References

1. Phelps DS, Umstead TM, Quintero OA, Yengo CM, Floros J (2011) In vivo rescue of alveolar macrophages from SP-A knockout mice with exogenous SP-A nearly restores a wild type intracellular proteome; actin involvement. Proteome Sci 9: 67.
2. Crouch E, Wright JR (2001) Surfactant proteins a and d and pulmonary host defense. Annu Rev Physiol 63: 521-554.
3. Mikerov AN, Umstead TM, Gan X, Huang W, Guo X, et al. (2008) Impact of ozone exposure on the phagocytic activity of human surfactant protein A (SP-A) and SP-A variants. Am J Physiol Lung Cell Mol Physiol 294: L121-130.
4. Kaksonen M, Toret CP, Drubin DG (2006) Harnessing actin dynamics for clathrin-mediated endocytosis. Nat Rev Mol Cell Biol 7: 404-414.
5. Jain D, Dodia C, Fisher AB, Bates SR (2005) Pathways for clearance of surfactant protein A from the lung. Am J Physiol Lung Cell Mol Physiol 289: L1011-1018.
6. Crowther JE, Schlesinger LS (2006) Endocytic pathway for surfactant protein A in human macrophages: binding, clathrin-mediated uptake, and trafficking through the endolysosomal pathway. Am J Physiol Lung Cell Mol Physiol 290: L334-342.
7. Breuiller-Fouché M, Dubois O, Sediki M, Garcia-Verdugo I, Palaniyar N, et al. (2010) Secreted surfactant protein A from fetal membranes induces stress fibers in cultured human myometrial cells. Am J Physiol Endocrinol Metab. 298:E1188-197.
8. Kustermans G, El Mjiyad N, Horion J, Jacobs N, Piette J, et al. (2008) Actin cytoskeleton differentially modulates NF-kappaB-mediated IL-8 expression in myelomonocytic cells. Biochem Pharmacol 76: 1214-1228.
9. Kang M, Kobayashi A, Wakabayashi N, Kim SG, Yamamoto M (2004) Scaffolding of Keap1 to the actin cytoskeleton controls the function of Nrf2 as key regulator of cytoprotective phase 2 genes. Proc Natl Acad Sci U S A 101: 2046-2051.
10. Mikerov AN, Haque R, Gan X, Guo X, Phelps DS, et al. (2008) Ablation of SP-A has a negative impact on the susceptibility of mice to Klebsiella pneumoniae infection after ozone exposure: sex differences. Respir Res 9: 77.
11. Silveyra P, Floros J (2012) Genetic variant associations of human SP-A and SP-D with acute and chronic lung injury. Front Biosci (Landmark Ed) 17: 407-429.
12. Floros J, Wang G, Mikerov AN (2009) Genetic complexity of the human innate host defense molecules, surfactant protein A1 (SP-A1) and SP-A2--impact on function. Crit Rev Eukaryot Gene Expr 19: 125-137.
13. Wang G, Bates-Kenney SR, Tao JQ, Phelps DS, Floros J (2004) Differences in biochemical properties and in biological function between human SP-A1 and SP-A2 variants, and the impact of ozone-induced oxidation. Biochemistry. 43:4227-4239.
14. Wang G, Guo X, Diangelo S, Thomas NJ, Floros J (2010) Humanized SFTPA1 and SFTPA2 transgenic mice reveal functional divergence of SP-A1 and SP-A2: formation of tubular myelin in vivo requires both gene products. J Biol Chem 285: 11998-12010.
15. Sánchez-Barbero F1, Strassner J, García-Cañero R, Steinhilber W, Casals C (2005) Role of the degree of oligomerization in the structure and function of human surfactant protein A. J Biol Chem 280: 7659-7670.
16. García-Verdugo I, Wang G, Floros J, Casals C (2002) Structural analysis and lipid-binding properties of recombinant human surfactant protein a derived from one or both genes. Biochemistry. 41:14041-14053.
17. Wang G, Taneva S, Keough KM, Floros J (2007) Differential effects of human SP-A1 and SP-A2 variants on phospholipid monolayers containing surfactant protein B. Biochim Biophys Acta 1768: 2060-2069.
18. Oberley RE, Snyder JM (2003) Recombinant human SP-A1 and SP-A2 proteins have different carbohydrate-binding characteristics. Am J Physiol Lung Cell Mol Physiol 284: L871-881.
19. Wang G, Umstead TM, Phelps DS, Al-Mondhiry H, Floros J (2002) The effect of ozone exposure on the ability of human surfactant protein a variants to stimulate cytokine production. Environ Health Perspect. 110:79-84
20. Huang W, Wang G, Phelps DS, Al-Mondhiry H, Floros J (2004) Human SP-A genetic variants and bleomycin-induced cytokine production by THP-1 cells: effect of ozone-induced SP-A oxidation. Am J Physiol Lung Cell Mol Physiol 286: L546-553.
21. Mikerov AN, Umstead TM, Huang W, Liu W, Phelps DS, et al. (2005) SP-A1 and SP-A2 variants differentially enhance association of Pseudomonas aeruginosa with rat alveolar macrophages. Am J Physiol Lung Cell Mol Physiol 288: L150-158.
22. Mikerov AN, Wang G, Umstead TM, Zacharatos M, Thomas NJ, et al. (2007) Surfactant protein A2 (SP-A2) variants expressed in CHO cells stimulate phagocytosis of Pseudomonas aeruginosa more than do SP-A1 variants. Infect Immun 75: 1403-1412.
23. Lopez-Rodriguez E, Pascual A, Floros J, Perez-Gil J (2013) Interfacial Behavior of Murine Pulmonary Surfactant Expressing Different Human Surfactant Protein SP-A Variants. Biophysical Society; Philadelphia, PA, USA.
24. Phelps DS, Umstead TM1, Silveyra P1, Hu S1, Wang G1, et al. (2013) Differences in the alveolar macrophage proteome in transgenic mice expressing human SP-A1 and SP-A2. J Proteom Genom Res 1: 2-26.
25. Bersani I, Speer CP, Kunzmann S (2012) Surfactant proteins A and D in pulmonary diseases of preterm infants. Expert Rev Anti Infect Ther 10: 573-584.
26. Vento G, Tana M, Tirone C, Aurilia C, Lio A, et al. (2012) Effectiveness of treatment with surfactant in premature infants with respiratory failure and pulmonary infection. Acta Biomed 83 Suppl 1: 33-36.
27. Herting E, Gefeller O, Land M, van Sonderen L, Harms K, et al. (2000) Surfactant treatment of neonates with respiratory failure and group B streptococcal infection. Members of the Collaborative European Multicenter Study Group. Pediatrics. 106:957-964.
28. Donn SM, Dalton J (2009) Surfactant replacement therapy in the neonate: beyond respiratory distress syndrome. Respir Care 54: 1203-1208.
29. Tan K, Lai NM, Sharma A (2012) Surfactant for bacterial pneumonia in late preterm and term infants. Cochrane Database Syst Rev 2: CD008155.

30. Noutsios GT, Silveyra P, Bhatti F, Floros J (2013) Exon B of human surfactant protein A2 mRNA, alone or within its surrounding sequences, interacts with 14-3-3; role of cis-elements and secondary structure. Am J Physiol Lung Cell Mol Physiol 304: L722-735.

31. Silveyra P, Wang G, Floros J (2010) Human SP-A1 (SFTPA1) variant-specific 3' UTRs and poly(A) tail differentially affect the in vitro translation of a reporter gene. Am J Physiol Lung Cell Mol Physiol 299: L523-534.