



# Editorial: Odyssey of Surfactant Proteins SP-A and SP-D: Innate Immune Surveillance Molecules

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## Editorial on the Research Topic

### Odyssey of Surfactant Proteins SP-A and SP-D: Innate Immune Surveillance Molecules

Surfactant protein A (SP-A) and D (SP-D) are hydrophilic collagenous C-type lectins, which were originally discovered in the lungs associated with surfactant phospholipids. It was later shown that the two proteins, unlike hydrophobic surfactant proteins, SP-B and SP-C, are keenly involved in protecting lungs against insults from pathogens, allergens, apoptotic, and necrotic cells (1). Two aspects became clear in subsequent years that (i). SP-A and SP-D have extra-pulmonary existence; and (ii). They can manipulate immune cells, and thus, regulate inflammatory responses (2). Although there have been a constant debate about their candidate receptor(s)—there are several reported so far (1). Much of the immunological studies, beyond interaction with surfactant system and pathogens, have been followed up toward SP-D. It has become apparent that SP-D is an innate immune surveillance molecule at the mucosal surfaces, which can act as a bridge between innate and adaptive immunity. The role of SP-D in modulating antigen presentation, helper T cell polarization and B cell differentiation and class switching (3) are few neat examples. This volume comprises 14 papers that extend our knowledge on SP-A and SP-D, and their roles in infection, inflammation and cancer.

A consistent theme discussed by several contributors is the differential role of two forms of SP-A in oxidative stress and lung innate immunity (Thorenoor, Umstead et al.; Nalian et al.; Thorenoor, Kawasaki et al.; Wang et al.). In humans, there are two SP-A variants differing in the collagen region, SP-A1 and SP-A2, encoded by SFTPA1 and SFTPA2, respectively, and produced by the alveolar type II cells in the lung. Importantly, SP-A1 and SP-A2 seem to differentially bind to phagocytic, but not to non-phagocytic cells (Thorenoor, Umstead et al.). SP-A1 and SP-A2 differentially bind and regulate neonatal and adult human alveolar macrophages (AMs) (Thorenoor, Umstead et al.). AMs from transgenic mice expressing human SP-A1 and SP-A2 exhibit differential expression of cell surface proteins (Thorenoor, Kawasaki et al.) Rodents express only one SP-A variant; thus, Nalian et al. have compared the rodent and human SP-A with respect to structural determinants of the function. The data infers that mouse SP-A is a functional hybrid of human SP-A1 and SP-A2. Particularly striking in this regard is the differential response in the two sexes. Humanized transgenic (hTG) male and female mice, carrying both SP-A1/SP-A2 (6A2/1A0, co-expressed) and SP-A-gene deficient mice were exposed to filtered air (FA) or ozone (O<sub>3</sub>), and miRNA levels were measured in isolated AMs (Thorenoor, Kawasaki et al.). The AM miRNome of co-expressed females was significantly downregulated in response to ozone induced oxidative stress. Several of the validated miRNA targets were involved in pro-inflammatory response, anti-apoptosis, cell cycle, cellular growth, and proliferation (Thorenoor, Kawasaki et al.). Continuing with this

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theme, Wang et al. have analyzed bronchoalveolar lavage (BAL) proteomic profile and associated signaling pathways in hTG SP-A1 and SP-A2 mice, as well as in SP-A knock-out mice exposed to O<sub>3</sub> or *Klebsiella pneumoniae*. The hTG-SP-A2 mice showed significantly higher number of differentially expressed proteins, with the majority being increased in male mice while decreased in female mice. Survival of hTG mice (expressing SP-A1 alleles/SP-A2 alleles/ Co-ex) challenged with *Klebsiella pneumoniae* was observed to be gene specific (co-ex and SP-A2 showing higher survival), variant-specific [co-expressed hTG (6A2/1A0) and hTG (1A0)] showing higher survival and sex specific (females showing higher survival). Cystic Fibrosis (CF) is characterized by altered SP levels. Lin et al. demonstrated that complex single nucleotide polymorphisms (SNPs)-SNP interactions of the surfactant genes may contribute to the pulmonary disease in CF patients. Wang Group have looked into SP-D gene polymorphisms and susceptibility to tuberculosis, by identifying/cloning two major SP-D exonic polymorphisms and examining their interaction with *Mycobacterium bovis* bacillus Calmette-Guérin (*M. bovis*) BCG. The authors seem to suggest that C92T (rs721917; amino acid rSP-D 92T variant) may increase susceptibility to TB (Hsieh et al.).

Hartshorn group have elegantly reviewed the molecular mechanisms used by SP-D to neutralize influenza A Virus (IAV) (Hsieh et al.). The group has been a pioneer in the field and the review summarizes the highlights of their credible work over a couple of decades. Al-Ahdal et al. have shown that a recombinant form of truncated human SP-D (rfhSP-D), composed of homotrimeric neck and C-type lectin domains, was able to restrict H1N1 and H3N2 subtypes of IAV (as well as their pseudotyped viral counterparts) from infecting A549 cells, a lung epithelial cell line, thus acting as an entry inhibitor. This is in contrast to the recombinant fragment of human SP-A that seems to promote IAV infectivity; however, full-length SP-A inhibits viral entry (4). These results seem to suggest that the two molecules i.e., SP-A and SP-D, especially their C-type lectin domains, can be very distinct in their functional properties; this point has been emphasized by other papers in this volume. One important point that needs mentioning is that rfhSP-D is also able to dampen the pro-inflammatory cytokine storm induced by IAV, which could minimize the lung injury caused by the virus. Next two papers are focused on how SP-D (rather rfhSP-D) can offer protection against HIV-1 at the mucosal surface. Dodagatta-Marri et al. show, for the first time, that DC-SIGN is a putative receptor for SP-D; the binding takes place via the C-type lectin domains of both proteins. This interaction is interestingly poised since both SP-D and DC-SIGN can interact with HIV-1. The authors show that opsonizing HIV-1 with rfhSP-D prior to viral exposure to DC-SIGN bearing cells reduced viral ability to get transferred to CD4<sup>+</sup> T cells *in trans*. Thus, this is another layer of protection that rfhSP-D works at against HIV-1, and follows up earlier studies (5, 6). A seminal study by Pandit et al. in this volume reports use of vaginal explants and inhibition of HIV-1 transfer in an *ex vivo* context. The authors have also carried out a transcriptomics analysis revealing how rfhSP-D modulates a wide range of target cells and pathways in order to act as a mucosal barrier to HIV-1. In addition, the

study has utilised a rabbit model of vaginal irritation in order to demonstrate that rfhSP-D is a safe prophylactic molecule for vaginal use.

Madan et al. showed nearly 20 years ago that rfhSP-D could offer therapeutic protection in a murine model of pulmonary hypersensitivity induced by *Aspergillus fumigatus* allergens/antigens (7). Subsequently, SP-D knock-out mice were found to be hyper-eosinophilic that could be ameliorated by rfhSP-D intranasal treatment (8). The mechanism of eosinophil clearance remained unclear until Mahajan et al. showed that eosinophils derived from allergic patients were susceptible to apoptosis induction by rfhSP-D (9) via p53 pathway, as revealed by proteomics analysis of a eosinophilic leukemic cell line, AML14.3D10 (10). This opened the area of SP-D mediated immune surveillance in cancer. It was subsequently shown that SP-D binds to EGF receptor on A549 cells and had an anti-proliferative and anti-invasive effect (11). In this volume, Kaur, Riaz, Murugaiah et al. reported the ability of rfhSP-D to induce apoptosis *via* TNF- $\alpha$ /Fas-mediated pathway regardless of the p53 status in human pancreatic adenocarcinoma (PDAC) using Panc-1 (p53<sup>mt</sup>), MiaPaCa-2 (p53<sup>mt</sup>), and Capan-2 (p53<sup>wt</sup>) cell lines. Treatment of these cell lines with rfhSP-D caused growth arrest in G1 cell cycle phase, triggered transcriptional upregulation of pro-apoptotic factors such as TNF- $\alpha$ , and induced apoptosis *via* Fas-mediated pathway in a p53-independent manner. In another paper, Kaur, Riaz, Singh et al. demonstrate that rfhSP-D is also capable of inhibiting TGF- $\beta$  expression in the PDAC cell lines, and thus, suppressing their invasive-mesenchymal properties. The rfhSP-D-treated pancreatic cancer cell lines showed reduced expression in the cytoplasm of Smad2/3, suggesting that an interrupted signal transduction negatively affected the transcription of key mesenchymal genes. Thus, expressions of Vimentin, Zeb1, and Snail were found to be downregulated upon rfhSP-D treatment. Both these studies suggest that rfhSP-D can potentially be used to therapeutically target pancreatic cancer cells. A bioinformatics analysis using Oncomine dataset and the survival analysis platforms Kaplan-Meier plotter has been performed by Mangogna et al., which showed that in the lung, gastric, and breast cancers, there is a lower expression of SP-D than normal tissues. On the contrary, a higher expression than normal tissue was observed in ovarian cancer. In lung cancer, the presence of SP-D could be associated with a favorable prognosis. On the contrary, at non-pulmonary sites such as gastric, breast, and ovarian cancers, the presence of SP-D could be associated with unfavorable prognosis. All these data indicate that SP-D could also be used as a potential diagnostic marker.

In a critical assessment, Colmorten et al. have reviewed the role of SP-D in the vascular inflammation, inferring a dual role of SP-D in the development of atherosclerosis. A pro-atherogenic role of SP-D is evident from *in vivo* studies in SP-D knock-out mice. Clinical studies have shown a positive association between circulatory SP-D levels, carotid intima-media thickness, coronary artery calcification and risk of both total and cardiovascular disease mortality.

It is clear from the studies being reported in this volume that the field of SP-A and SP-D continues to grow and is

now throwing a number of pleasant surprises. The therapeutic potential of rfhSP-D needs to be realized via planned clinical trials. This decade is going to be an exciting one for surfactant protein research.

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## AUTHOR CONTRIBUTIONS

All authors listed have made an equal, direct and intellectual contribution to the work, and approved it for publication.

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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