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Human surfactant protein A (SP-A) variants: why so many, why such a complexity?

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Human surfactant protein A (SP-A) exhibits extensive complexity at several levels: genetic, transcript (splicing), protein, and composition and size of protein oligomers. Its multiple and important roles in innate host defense, regulation of inflammation, and in aspects of pulmonary surfactant may have necessitated such a complexity from an evolutionary point of view. Moreover, understanding of such a complexity may be useful in the study of disease pathogenesis and the development of disease diagnostics and/or therapeutics.

SP-A

Originally SP-A was identified as a surfactant associated protein and studied for its role in surfactant function and/or biology (i.e. structure of surfactant). Following cloning of genomic DNA [1] and two cDNA sequences [2], a structural similarity between SP-A and acute phase reactant molecules such as the mannose binding protein (MBP) and C1q was observed [3].

Although no sequence similarity exists among MBP, C1q, and SP-A, the structural similarity opened up the possibility of a role of SP-A in host defense in the lung. After more than a decade of work, it is currently well established that SP-A plays a major role in innate host defense and the regulation of inflammatory processes in the lung. Expression of SP-A has been observed in tissues other than lung, suggesting that the role of SP-A in host defense is not limited to the lung [4, 5].

Human SP-A genes and alleles

In contrast to rodents, humans [1, 6] and primates [7] have two SP-A genes. In humans, these may have arisen by gene duplication [8], and are found in opposite transcriptional orientation with a pseudogene sequence in the middle on the long arm of chromosome 10 [9–11]. For each human SP-A gene, based on sequence differences within the coding region, more than 30 genetic variants (alleles) have been fully or partially characterized [12]. Of these four SP-A1 (6A, $6A^2$, $6A^3$, $6A^4$) (Fig. 1B) and six SP-A2 (1A, $1A^0$, $1A^1$, $1A^2$, $1A^3$, $1A^5$) (Fig. 2B) alleles are observed frequently (>1%) in the general population [12]. The SP-A1 alleles differ at 5 codons, encoding amino acids 19, 50, 62, 133, 219 (Fig. 1A) and the SP-A2 alleles at codons for amino acids 9, 91, 140, and 223 (Fig. 2A).

Several 5′ untranslated exons (A, B, C, D) exist (Fig. 3) that splice in different configurations to give, for the most part, SP-A1 and SP-A2 specific transcripts. The major SP-A2 transcripts are ABD and ABD′. The ABD has 3 more nucleotides than the ABD′ transcripts. Alleles $1A^0$ and $1A^1$ have two potential splice sites generating both ABD and ABD′ transcripts, whereas alleles 1A and $1A^2$ can only generate the ABD′ transcript due to a nucleotide difference within the consensus splice sequence. The major SP-A1 transcript is the AD′. Minor transcripts for SP-A1 (ABD′, ACD′) and other rare transcripts for both SP-A1 and SP-A2 have also been identified [13]. Differences within the 3′ untranslated region of the SP-A sequences exist [2, 6, 14, 15] and these differences may play a role in the regulation of SP-A (see below).

Therefore, the SP-A variants hold the potential for qualitative (functional) differences due to nucleotide differences within the coding sequence (Figs 1 and 2) that may result in a change of the encoded amino acid and also hold the potential for quantitative (regulatory) differences due to 5′ UTR splice variants (Fig. 3) and to 3′ UTR sequence differences.

Figure 1

Human SP-A1 alleles: nucleotide differences and frequencies. Panel A denotes the most frequently observed SP-A1 alleles (6A, $6A^2$, $6A^3$, $6A^4$) and the nucleotide differences among these alleles. These differences are observed in codons for amino acids (AA) 19 (GC/TG), 50 (C/GTC), 62(CCG/A), 133 (ACG/A), and 219(C/TGG). The overall pattern of differences at the indicated locations determines each SP-A1 allele. Panel B depicts the frequency of the most commonly observed SP-A1 alleles in the general population (n>2000) [12].

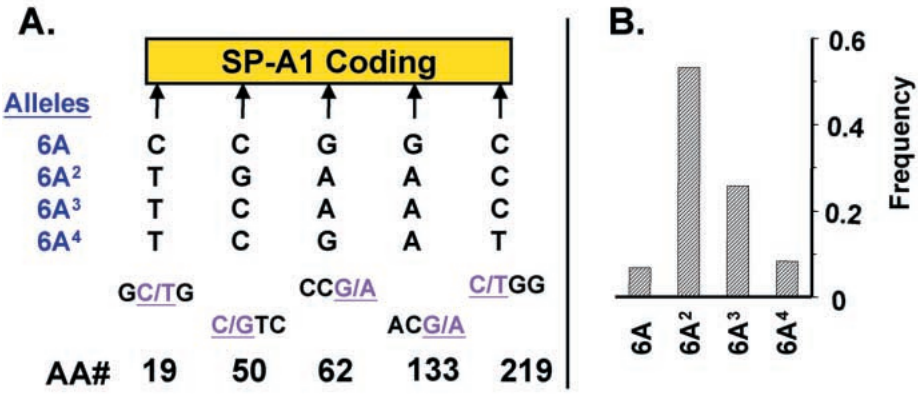


Figure 2

Human SP-A2 alleles: nucleotide differences and frequencies. Panel A denotes the most frequently observed SP-A2 alleles (1A, $1A^0$, $1A^1$, $1A^3$, $1A^5$) and the nucleotide differences among these alleles. These differences are observed in codons for amino acids (AA) 9 (AA/CC), 91 (C/GCT), 140 (TCC/T) and 223 (C/AAG). The overall pattern of differences at the indicated locations determines each SP-A2 allele. Panel B depicts the frequency of the most commonly observed SP-A2 alleles in the general population (n>2000) [12].

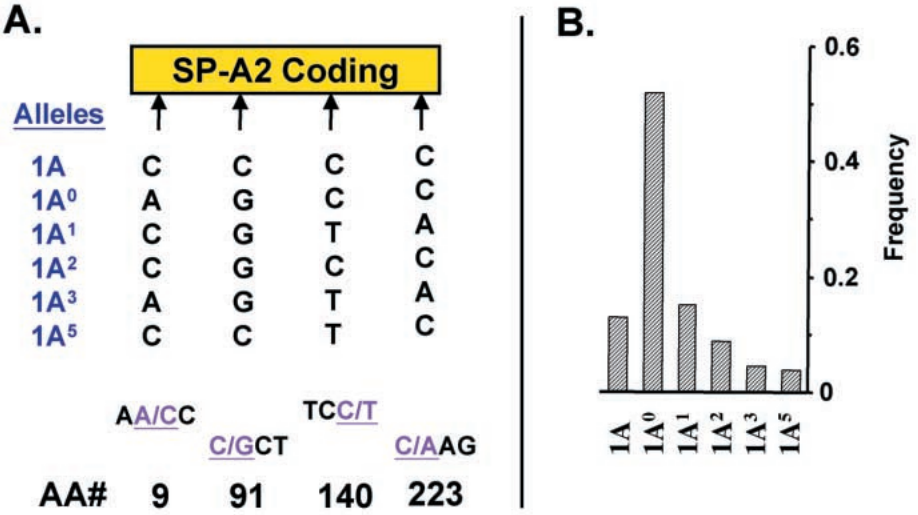
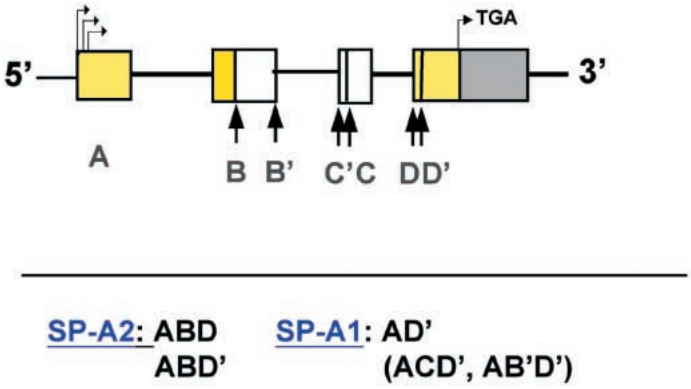


Figure 3

Human SP-A 5′ UTR and splice variants. There are several 5′ untranslated exons (A, B/B′, C′/C, and D/D′) in both SP-A1 and SP-A2. These exons splice in different configurations specific for SP-A2 (ABD and ABD′: major transcripts) and for SP-A1 (AD′: major transcript; ACD′ and AB′D′ minor transcripts). The translation start site (TGA) is noted. The arrows in exon A denote different transcription start sites [17]. Not drawn to scale.



Functional and regulatory differences among SP-A variants

Native SP-A is biochemically complex, as its precursor molecules undergo several co- and post-translational modifications to give rise to isoforms with differences in the isoelectric point and the molecular weight (Fig. 4). SP-A from bronchoalveolar lavage contains both SP-A1 and SP-A2 gene products, and in the early 90s [16] it was suggested that SP-A consists of two SP-A1 molecules and one SP-A2 forming a trimer, and that six trimers give rise to a dodecamer. This 2:1 ratio of SP-A1 to SP-A2 suggested that the level of SP-A1 protein should be twice that of SP-A2. Studies of SP-A mRNA have shown that the SP-A1 to SP-A2 ratio varies beyond the proposed 2:1 ratio, suggesting that single gene products (if mRNA reflects protein levels) are present [17]. Whether the single gene products are functional or harmful, and/or whether differences exist among SP-A1, SP-A2, and SP-A proteins that include both gene products is not entirely known.

Recent findings indicate that although single gene products are functional with regard to

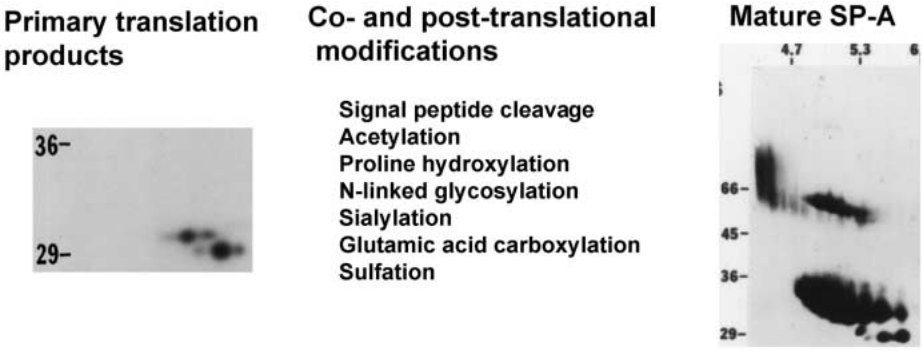


Figure 4

Two-dimensional gel electrophoresis of human SP-A precursor molecules and native SP-A from bronchoalveolar lavage (BAL). The two precursor molecules (major spots) of 29 and 31 kDa [23] and their isoforms (minor spots), which are due to acetylation, are depicted in the left panel. Cotranslational and posttranscriptional modifications (listed in the middle) of the precursor molecules, give rise to native SP-A consisting of multiple isoforms in molecular weight and isoelectric points shown in the right panel (monomers at ~35 kDa, dimers at ~60–65 kDa etc).

their ability to enhance TNF-α production by a macrophage-like cell line, significant differences between SP-A1 and SP-A2 variants in the level of enhancement of TNF-α production are observed [18]. Moreover, the ability of in vitro expressed SP-A variants to stimulate TNF-α production is reduced if the variants are exposed to ozone [19]. Also, differences in the level of TNF-α production among ozone-exposed SP-A1 alleles are observed [19].

Regulatory differences in response to dexamethasone among SP-A alleles have been observed [20]. Also, differences among SP-A alleles in basal levels have been observed, as have 5′ UTR-dependent differences in the efficiency of translation between SP-A1 and SP-A2 alleles (our unpublished observations).

The available information indicates that genotype-dependent quantitative and qualitative differences may exist among human SP-A variants.

Why such a complexity?

From an evolutionary point of view I would like to put forward the thought that perhaps the role of SP-A in innate host defense has necessitated the evolution of such complexity. As the space occupied (traveled) by a species moving up the evolutionary ladder increased, contact with a larger number of potentially harmful agents also increased, necessitating perhaps evolutionary modification of the regulatory and functional capabilities of SP-A in order to adapt to new environmental threats. The large number of genetic variants and splice variants that have been characterized in humans may indeed reflect the result of such an adaptation.

In relevance to this putative adaptation the splice complexity at the 5′ UTR (Fig. 3) is rather intriguing and may exemplify a form of genetic parsimony. This type of genetic economy may assure stability of the gene, while a response to additional and/or novel stimuli is accommodated partly via intermediary forms (i.e. splice transcripts). Perhaps yet another example of the ultimate ingenuity of Mother Nature.

SP-A complexity and disease

Increased or decreased SP-A has been associated with several pulmonary diseases [21]. Oligomer size has also been associated with disease [22]. However, the way in which total SP-A levels are currently measured does not provide a marker specific for a given disease. It is possible that SP-A complexity can provide the basis for the development of diagnostic tests specific for each pulmonary disease. For example, although it has been hard to grow antibodies specific for the SP-A1 and SP-A2 products (our unpublished observations), the development of specific SP-A1 and SP-A2 antibodies could help identify the relative levels of each SP-A gene. The specific SP-A1 to SP-A2 ratio along with the pattern of oligomer composition and size, i.e. whether SP-A oligomers consist of both or single gene products, and/or the relative ratio of different size oligomers in health or disease may help develop a matrix profile. This matrix profile could serve as a diagnostic tool with a high degree of disease specificity. It could also help in the choice of therapeutic treatment strategies. In addition, with further advances in basic scientific knowledge, the specific matrix profile may help gain insight into disease pathogenesis and develop reagents to disrupt specific SP-A interaction when such interaction is deemed deleterious.

Final thoughts

The diversity and the level of complexity we have come to recognize and begin to understand among SP-A variants are likely to reflect a "window" view of much more complexity to be unraveled. Since the mid-80s, with the characterization of the SP-A precursor molecules and the cloning of these molecules and the corresponding genomic sequences, study of the human SP-A system has been scientifically rich as new and unexpected directions of study continually unfold. SP-A, although it is not essential for life, may be very important in the qualitative aspects of life given its multiple known roles and perhaps others yet to be discovered. Currently, SP-A because of its role in innate host defense, the regulation of inflammatory processes or its role in surfactant structure and/or function, and the central importance of these functions and/or processes in most (if not all) pulmonary diseases, holds the potential to contribute to overall health and/or disease pathogenesis in the lung. Thus, SP-A may serve as a good model for the study of pulmonary disease pathogenesis.

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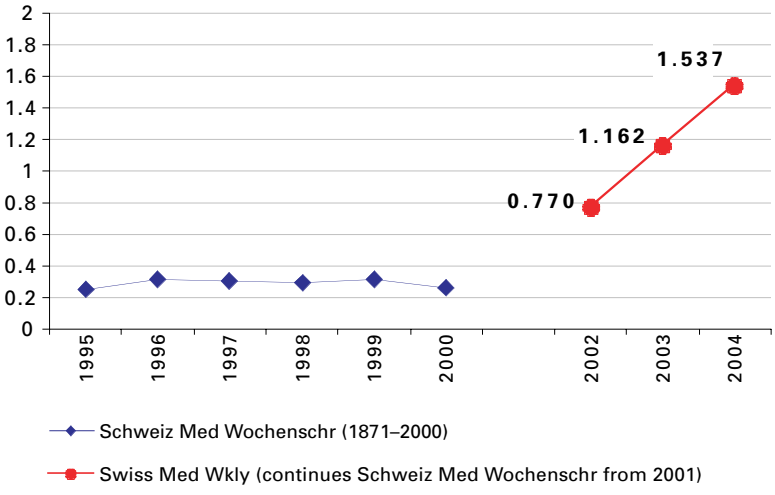

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