Between adaptation and virulence

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Chapter 6

Summary and future perspectives

Staphylococcus aureus is one of the commonly encountered bacteria of the human microbiome. Although mostly a seemingly harmless commensal microbe, *S. aureus* can act as an invasive pathogen with seriously devastating effects on its host's health and wellbeing. A wide range of infections caused by this bacterium has been reported to affect diverse parts of the human body, including the skin, soft tissues and bones, as well as important organs like the heart, kidneys and lungs. Particularly, *S. aureus* is infamous for being a major causative agent of respiratory tract infections that may escalate up to necrotizing pneumonia. Due to its clinical relevance, this pathogen has been intensively studied for many years. Nonetheless, further research in this field is still needed, because of the high capacity of *S. aureus* to evolve drug resistance, its high genomic plasticity and adaptability and, not in the last place, the plethora of niches within the human body where it can thrive and survive. In this regard, there are still many uncertainties concerning the specific adaptations carried out by *S. aureus* during colonization and infection of the human body, the transition between both stages, and upon the invasion of different types of host cells. To shed more light on some of these adaptations, the research described in this thesis has employed *in vitro* models of infection that mimic particular conditions during the infectious process with special focus on the lung epithelium. The adaptations displayed by *S. aureus* were monitored using advanced proteomics. Furthermore, the analyses documented in this thesis included *S. aureus* strains with diverse backgrounds and epidemiology to take into account the genetic diversity encountered in this species.

A general introduction to the current status of the field is presented in **chapter 1**, which highlights the genomic plasticity of *S. aureus* and its capability to optimally regulate its gene expression for propagation and survival in the challenging ecological niches of the human body. Of note, the genomic variability of *S. aureus* is based on its capability to acquire mobile genetic elements, which often carry

genes granting antibiotic resistances and encoding virulence factors. In particular, chapter 1 showcases the virulence factors and regulatory mechanisms employed by *S. aureus* to conquer and corrupt the cells, tissues and immune defenses of the human host. Additionally, the distinction between community- and hospital-acquired Methicillin resistant *S. aureus* (CA- and HA-MRSA) is introduced.

The adaptive behavior of *S. aureus* during infection in specific niches of the human body is still not completely understood, and this applies particularly to its intracellular residence over extended periods of time. For this reason, the research described in **chapter 2** was undertaken with a focus on the dynamic relationship between *S. aureus* and lung epithelial cells. Over 4 days post internalization the proteomes of host cells and the invading pathogen were monitored, revealing a continuous bidirectional interaction. Of note, during the infection, two subpopulations of *S. aureus* displaying differential rates of replication and intracellular localization were observed. A replicating subpopulation was more abundant during the first 24 h of infection, remained enclosed in vesicles, and displayed a rapid increase in numbers, which culminated in the lysis of the host cells. The proteomics analyses illustrated that this lytic event was an apoptotic reaction, most likely caused by the presence of the pathogen intracellularly. The other subpopulation of internalized *S. aureus*, which displayed a dormant phenotype, was found to reside predominantly in the host cytosol. By the end of the experimental time window of four days, the presence of this dormant *S. aureus* population correlated with the expression of inflammatory host proteins. Furthermore, the simultaneous proteomic inspection of host and pathogen revealed a continuous interplay at the metabolic level, which potentially determines the outcome of the infection. In this regard, the pathogen was found to regulate expression of proteins related to an exposure to nutrient- and oxygen-deprived environments. For example, the presumably microaerobic intracellular conditions triggered an increase in the production of proteins related to

fermentation. Further significant changes involved the central carbon metabolism, where an increase in proteins related to the TCA cycle and amino acid degradation was observed. These proteomic changes relating to metabolic pathways reflect the pathogen's efforts to optimize energy production from alternative carbon and nitrogen sources. Moreover, the presumptive depletion of amino acids by the pathogen will have an impact on the host metabolism, in particular the arginine and asparagine biosynthetic pathways, as previously reported (1). Importantly, the investigations detailed in chapter 2 show that also other amino acid metabolic pathways may have an impact on host-pathogen interactions, especially those for proline, glutamate and alanine. Taken together, the documented results highlight the interplay between host and pathogen at the metabolic level and their reciprocal adaptations. Ultimately, the host cells that survived the infection carried a non-replicating persistent population of *S. aureus* in their cytoplasm. These findings show that the final outcome of an intracellular *S. aureus* infection is not only determined by the production of virulence factors, but also by the usage of intracellular resources, and the subsequent metabolic adaptations by the host and its intracellular bacteria.

The analyses described in chapter 2 do not consider the potential variations occurring when there is an underlying disease condition. For this reason, the implemented lung epithelial model was adapted to represent two regenerative stages of the lung epithelium, namely the initial stage of migration and repair, and the subsequent stage of fibrogenesis. As described in **chapter 3**, the results from this study demonstrated that initial stages in the regeneration of the host epithelium have a significant impact on the infection dynamics. The low polarization level observed during the migratory state of the epithelial cells permitted higher internalization and intracellular replication rates of *S. aureus*, which led to reduced host cell survival. In contrast, the fibrogenesis stage displayed a strengthening of the tight junctions, thereby reducing internalization

of the pathogen and leading to low bacterial replication rates upon internalization. Regardless of the differences observed for these two infection settings, the adaptations of the bacterial proteomes to the intracellular conditions were found to be very similar. Nevertheless, differences were detected for *S. aureus* proteins regulated by the Rex regulon. Rex senses imbalances in the redox state of the bacteria, which could be caused by the levels of available oxygen, activation of the TCA cycle, or an abundance of NO. Further analyses showed that only the NO levels were different between the two infection settings, and that this was a consequence of the overproduction of the NO synthetase of *S. aureus*, a phenotype that can be correlated with high colonization rates (2). These observations imply a NO-dependent modulation of the bacterial cytoplasmic redox state to maintain homeostasis prior to internalization. Altogether, the studies described in chapter 3 provide a deeper insight into how *S. aureus* takes advantage of a breached epithelial barrier, and how infected epithelial cells have a limited ability to respond adequately to staphylococcal insults.

To expand the analysis of *S. aureus* adaptability to the host environment, the last two experimental chapters of this thesis address MRSA isolates from different epidemiological backgrounds. **Chapter 4** documents genomics and proteomics analyses to characterize and differentiate two groups of 6 isolates each, representing CA- and HA-MRSA variants of the *S. aureus* USA300 lineage collected in Denmark (DK). Additionally, this study included three *S. aureus* HA isolates from the Dutch-German border region (NL-DE) as benchmark. At the genetic level, the core genome of CA^{DK}-isolates presented more similarity to $HA^{NL\text{-}DE}$-isolates, while the accessory genome presented more similarities between the HA^{DK} and $HA^{NL\text{-}DE}$-isolates. At the exoproteome level, however, most identified proteins were shared among all groups. Nonetheless, differences were found in the amounts of proteins expressed by the different groups and in group-specific proteins. The vast majority of such differences was found to involve secreted

proteins with a predicted cytoplasmic localization, now referred to as extracellular cytoplasmic proteins or ECP (3). Most likely, at least some of these proteins have a moonlighting nature, serving not only their well-defined functions in the bacterial cytoplasm, but also extracellular functions in the colonization and infection of the host. Interestingly, a higher abundance of such proteins was observed for CA-isolates during the exponential growth phase and for HA-isolates during the stationary phase. This difference might be related to differential timing of ECP secretion events in the two epidemiologically distinct groups. Lastly, a comparison of exoproteome abundance signatures showed that, regardless of the distinct geographical origin of the investigated isolates, the two HA-groups cluster together. Subsequent internalization experiments using lung epithelial cells mirrored the clustering based on the exoproteome analyses. These findings focus special attention on possible roles of 'liberated' ECPs in the epidemiology and intracellular survival of CA- and HA-MRSA isolates. ECPs were already invoked in the virulence of *S. aureus*, but a possible role in the epidemiology of MRSA is new. Further, this study implies that proteomics could become a useful tool for characterizing *S. aureus* isolates and predicting their epidemiological behavior.

As concluded in chapter 2, metabolic adaptations of isolates play an important role as drivers of virulence. Accordingly, **chapter 5** presents an extended analysis of a subset of the clinical HA- and CA-MRSA isolates from Denmark investigated in the studies described in chapter 4. The cytosolic proteomes of this subset displayed significant differences between the two groups of isolates. The CA-MRSA group presented higher amounts of proteins related to the TCA cycle, amino acid metabolism and gluconeogenesis. This disposition of the CA-isolates towards these pathways underlines their preparedness to encounter nutrient-deficient environments, consistent with their clinical presentation in skin and soft tissue infections. Conversely, the HA-isolates displayed higher levels of proteins related to glycolysis, and the pentose phosphate pathway and lower levels of

proteins belonging to the purine biosynthetic pathway. This indicates that the HA-isolates may prefer niches with abundant resources, such as blood. Altogether, these observations support the view that adaptations in central carbon metabolism are key drivers that streamline the investigated MRSA isolates for infection of healthy individuals in the community or frail patients in the hospital.

In conclusion, the studies detailed in this dissertation highlight the importance of staphylococcal metabolism and fitness as pertinent drivers of virulence. Although metabolic pathways are frequently ignored in infection research, in fact, they influence the capability of *S. aureus* isolates to thrive and survive in a plethora of different host environments. Moreover, across the different studies presented in the current thesis, proteomics proved to be an invaluable tool to explore the adaptions of both *S. aureus* and its host during infection conditions, and to deepen our understanding of the differences among CA- and HA-MRSA isolates. The primary characterization of the investigated CA- and HA-MRSA isolates on a proteome level was based on cultures in RPMI medium, which represents a condition that mimics nutrient supply during blood stream infections (4). Nonetheless, this setting lacks the presence of human host cells, and it will therefore be of interest to continue the characterization of MRSA isolates with different epidemiological backgrounds by using infection models that are even more realistic representations of infection, such as the lung epithelial cell model used for the studies described in chapters 2 and 3 of this dissertation. Moreover, the human immune system comprises several types of cells and defense mechanisms that *S. aureus* must evade or eliminate in the course of an infection. Therefore, further studies should also consider models that include a more complex representation of the host, for example by the inclusion of both epithelial and immune cells in the infection model. Such multi-cell type infection models are likely to play increasingly prominent roles in infection research in the years to come, and they will provide us with a deeper understanding of the complex

networks of interactions between the human host and commensal pathogens like *S. aureus*.

References

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