Friday, March 22, Session V

L17

A MULTIFACETED ROLE OF FILAMENTOUS HEMAGGLUTININ (FHA) IN THE VIRULENCE OF PATHOGENIC *BORDETELLA* SPECIES

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Filamentous hemagglutinin (FHA), a major virulence factor of classical *Bordetella*e, is a rod-shaped molecule that plays an important role in the adherence of bacteria to ciliated epithelial cells of the upper respiratory tract and suppresses the host innate and adaptive immune response. FHA is translated as a 360-kDa FhaB precursor that is exported across the outer bacterial membrane by a two-partner secretion mechanism involving the outer membrane protein FhaC and shed into external environment as an N-terminal 'mature' 220-kDa FHA protein after processing by surface-exposed SphB1 protease. The remaining C-terminal 130-kDa FhaB prodomain is thought to regulate maturation process and rapidly degraded in the periplasm. We show here that both the extreme C terminus (ECT) of the FhaB prodomain and the mature FHA play the pivotal roles in the virulence of *B. pertussis*. The NMR-based structural analysis of ECT, a highly-conserved the C-terminal 100 residues of the FhaB precursor, revealed that the ECT polypeptide adopts a rigid structure with a 'pilin-like' protein fold. Deletion of the sequence encoding ECT (ECT) resulted in a significant decrease in bacterial colonization within the nasal cavity of infected mice, comparable to *B. pertussis* strain lacking the FhaB precursor (FhaB). Intriguingly, the ECT strain exhibited a complete loss of its ability to bind cilia on human nasal epithelial cells grown at the air-liquid interface, emphasizing the indispensable role of ECT in the adherence of Bordetella cells to ciliated epithelial cells. Furthermore, we demonstrate the mature FHA confers resistance of *B. pertussis* to complement-mediated killing, highlighting its involvement in protection of bacterial cells against the host's innate immune response. Collectively, these results provide novel insights into FHA biology, unraveling its multifaceted role in the virulence of pathogenic *Bordetellae*.

L18

STRUCTURAL BIOLOGY OF ACUTE MYELOID LEUKEMIA (AML) PROTEIN AND SMALL MOLECULE INHIBITOR COMPLEX

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Nanotemper

Acute myeloid leukemia (AML) is a malignant disease of immature myeloid cells and the most prevalent acute leukemia among adults. The oncogenic homo-tetrameric fusion protein RUNX1/ETO results from the chromosomal translocation t(8;21) and is found in AML patients. The nervy homology region 2 (NHR2) domain of ETO mediates tetramerization; this oligomerization is essential for oncogenic activity. Previously, we identified the first-in-class small-molecule inhibitor of NHR2 tetramer formation, **7.44**, which was shown to specifically interfere with NHR2, restore gene expression down-regulated by RUNX1/ETO, inhibit the proliferation of RUNX1/ETO-depending SKNO-1 cells, and reduce the RUNX1/ETO-related tumor growth in a mouse model. However, no biophysical and structural characterization of **7.44** binding to the NHR2 domain has been reported. Likewise, the compound has not been characterized as to physicochemical, pharmacokinetic, and toxicological properties. Here, we characterize the interaction between the NHR2 domain of RUNX1/ETO and **7.44** by biophysical assays and show that **7.44** interferes with NHR2 tetramer stability and leads to an increase in the dimer population of NHR2. The affinity of **7.44** with respect to binding to NHR2 is K_{lig} = 3.75 1.22 M. By NMR spectroscopy combined with molecular dynamics simulations, we show that **7.44** binds with both heteroaromatic moieties to NHR2 and interacts with or leads to conformational changes in the N-termini of the NHR2 tetramer. Finally, we demonstrate that **7.44** has favorable physicochemical, pharmacokinetic, and toxicological properties. Together with biochemical, cellular, and in vivo assessments, the results reveal **7.44** as a lead for further optimization towards targeted therapy of t(8;21) AML.

L19

STRUCTURAL CHARACTERIZATION OF THE INTERACTION BETWEEN BRCA1-BARD1 AND RNA POLYMERASE II

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Transcription – a crucial process, which primarily generates messenger RNAs – competes for its substrate, DNA, with other processes, such as DNA repair. However, the principles underlying the crosstalk between the abovementioned processes remain poorly understood. The existing evidence indicates that the BRCA1-BARD1 complex, an early factor in the DNA damage response, is among the potential candidates for coordinating transcription and DNA repair. In our study, we investigated the molecular mechanism of the interaction between RNA polymerase II (RNAPII) and the BRCA1-BARD1 complex, as well as its functional consequence. Our data suggest that the BRCT repeat of BRCA1 binds the CTD, phosphorylated on Ser5, via the established mechanism. Moreover, we show that the interaction between the BRCT repeats and the hyperphosphorylated CTD is crucial for organisation of RNAPII into condensates with liquid-like properties. A subsequent analysis of disease-associated variants identified within the BRCT repeats supports our view that the observed condensation is indeed biologically relevant. Altogether, our data suggest that the BRCA1-BARD1 complex may coordinate transcription and DNA repair by organising RNAPII into transcription factories.

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Jacek Patryn

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