

fibrotic effect is mediated by SIP₃ and that it relies on downstream activation of ERK1/2 and ERM (ezrin-radixin-moesin), since when these signaling pathways were pharmacologically blocked, the SIP action was significantly reduced. Taken together these findings add new information to the understanding of the molecular mechanisms involved in endometriosis pathogenesis and identify SIP₃ as possible new pharmacological target to counteract the fibrotic trait of the disease.

P-32-033

Studying the activation of blood coagulation induced by staphylococcal biofilms: unraveling the link between infections and thrombosis

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Infective endocarditis (IE) is an infection of the cardiac endothelium. It has an annual incidence of 3–10/100,000 of the population with a mortality of up to 30%. *Staphylococcus aureus* is the most prevalent cause of IE. After endothelial injury, bacterial colonisation is facilitated, thus triggering additional endothelial injury and thrombus formation. Production of a biofilm (multicellular community of microorganisms, enclosed in a self-produced extracellular matrix) assists bacterial persistence and contributes to antibiotic tolerance. Considered that thrombotic complications are often associated with IE, here we explore the possibility that biofilms produced by *Staphylococcus aureus* (coagulase +) and *Staphylococcus epidermidis* (coagulase -), could induce fibrin generation in human plasma and investigate their ability to directly convert fibrinogen into insoluble fibrin *in vitro*. *S. aureus* and *S. epidermidis* were cultured and added to a 96-well microtiter plate, leading to the formation of bacterial biofilms. To test fibrin generation in plasma, diluted (1:2) human plasma was added to each biofilm and to empty wells as a blank experiment. To test fibrin generation from purified fibrinogen, a solution of fibrinogen (0.68 mg/ml) was added to each biofilm and to empty wells as a control. In both assays, fibrin generation was monitored by turbidimetry. The data obtained indicate that: both biofilms of *S. aureus* and *S. epidermidis* efficiently and similarly induce fibrin clotting in human plasma; both biofilms of *S. aureus* and *S. epidermidis* do not convert isolated fibrinogen solutions into fibrin. The results of this study point out that bacterial biofilms can trigger blood coagulation, thus providing the molecular basis for explaining the positive relationship between IE, biofilm formation and an increased thrombotic risk. Further studies are needed to elucidate the biochemical mechanisms underlying biofilm-induced activation of blood coagulation.

P-32-034

Amyloid fibrils induce blood coagulation by activating FXII: pathogenesis of intracardiac thrombosis in TTR and AL amyloidosis

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Transthyretin amyloidosis (ATTR) and immunoglobulin light chain amyloidosis (AL) are amyloid diseases characterized by fibril deposition in several organs, associated with cardiomyopathy and intracardiac thrombosis. However, the link between amyloid diseases and coagulation is still unclear. We demonstrated ability of amyloid fibrils to induce plasma clotting, and we established the molecular mechanisms resulting in activation of blood coagulation. The effect of the net charge of the fibril components was investigated by comparing the effect of fibrils made with similar precursors carrying opposite net charges (λ I and λ 6). All fibrils (both from *ex vivo* specimens and from recombinant origin, characterized by ThT, DLS, TEM) were added to human plasma or whole blood, monitoring clotting by turbidimetry, Thrombin Generation Assay or Thromboelastometry. Enzymatic assays were performed with isolated zymogens and chromogenic substrates. Binding of coagulation factors to fibrils was quantified by ELISA. Our data indicate that both natural and artificial fibrils similarly induce clotting. Addition of either TTR in a non-fibrillar state or of the amorphous precipitate of β -casein does not trigger clotting, while λ I amyloid fibrils, carrying a positive net charge at pH 7.4, partially lose the ability to trigger blood clotting. All fibrils induce activation of Factor XII (FXII), both in the presence of Factor XI (FXI) and prekallikrein (PK). Fibrils are also able to increase the efficiency of Prothrombin (FII) activation by activated Factor X (FXa). Fibrils represent a surface on which FXII, FII, FV, and FXa can anchor and become activated. The ability to activate FXII and blood coagulation appears to be correlated with the net charge of the amyloid protein components, with negatively charged fibrils being the most efficient in triggering blood coagulation. These mechanisms can explain the hypercoagulable state that can lead to thrombosis in cardiac amyloidosis.

P-32-035

Induction of E-cadherin expression by deficiency of steroid sulfatase is a key factor in hyperkeratinization for X-linked ichthyosis

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X-linked ichthyosis (XLI) is a genetic disorder caused by a deficiency of steroid sulfatase (STS), leading to an excessive accumulation of cholesterol sulfate and excessive keratinization. The hyperkeratinization seen in STS knockout (KO) mice demonstrates that this model is suitable for studying XLI. Additionally, RNA-seq analysis of skin tissues from STS KO and transgenic

mice revealed an enrichment of genes associated with calcium signaling such as calcium-sensing receptors, and keratinocyte differentiation markers such as keratin 1 and keratin 10. In our studies in STS-deficient cells, we observed an upregulation of E-cadherin, involved in keratinocyte differentiation and stratification through calcium interaction, along with increased levels of the keratinized envelope proteins and keratinization markers such as involucrin and loricrin. Intriguingly, the downregulation of STS and the subsequent accumulation of cholesterol sulfate inhibited the expression of Hakai, a ubiquitin E3 ligase of E-cadherin, thus prohibiting E-cadherin endocytosis and ubiquitin degradation, thereby stabilizing E-cadherin. Furthermore, our finding indicated that the inhibition of E-cadherin led to decreased levels of involucrin and loricrin in HaCaT cells. Specifically, mutations occurring in the N-terminal region of the E-cadherin domain, which is crucial for cell adhesion and calcium interaction, significantly reduced the expression of involucrin and loricrin. Moreover, treatment with miR-6766, known to impede E-cadherin expression, resulted in the suppression of keratinization marker proteins. Consequently, STS deficiency activates the calcium signaling system, promoting keratinocyte differentiation, and fostering excessive keratinization via the E-cadherin upregulation. Therefore, E-cadherin emerges as an important key factor in the hyperkeratinization observed in XLI, and these findings provide valuable insight into potential therapeutic targets for addressing XLI.

P-32-036

Histone deacetylase 3 regulates the life and death cycle of adipocytes in white adipose tissue

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Understanding adipose tissue physiology is crucial in the context of obesity epidemic. Chemical inhibition or genetic inactivation of histone deacetylase 3 (HDAC3) causes metabolic rewiring of white adipocytes toward browning through a futile cycle of lipolysis and lipogenesis [Previously published in: Galmozzi A et al. (2013) *Diabetes* 62, 732–742; Ferrari A et al. (2017) *Nat Commun* 8, 93]. Current research focuses on phenotypic alterations in white adipose tissue (WAT) from HDAC3 KO (H3fatKO) mice with emphasis on the impact of enhanced fatty acid metabolism on adipocyte health. To achieve our aim, we conducted immunohistochemistry, RNAseq, ChIPseq (H3K27ac) and FACS analyses on WAT of H3fatKO mice and floxed control mice. Adipocytes in which HDAC3 was silenced were assayed for cell proliferation and gene expression. Surface marker analysis was performed on bone marrow macrophages (BMDM) co-cultured with adipocytes. Dead adipocytes surrounded by macrophages are present in the visceral WAT of H3fatKO mice. FACS analysis shows a 2.48-fold change increase ($p = 0.0311$) in infiltrating macrophages in WAT, which exhibit polarization towards an M2-like population. Moreover, HDAC3 silencing in cultured adipocytes prompts macrophage activation. Integrated analysis of transcriptomic and epigenomic data unveils ferroptosis as one of the enriched pathways in H3fatKO mice, suggesting ferroptosis as a mechanism for eliminating metabolically exhausted

adipocytes. Concurrently, evidence of progenitors undergoing differentiation supports a potential renewal process in WAT. Gene expression and FACS analyses validate increased adipocyte proliferation. In conclusion, HDAC3 ablation in adipocytes remodels WAT immunophenotype and may serve as a metabolic rheostat, influencing the rejuvenation of WAT by eliminating aging adipocytes and fostering progenitor differentiation. Insight into the role of HDAC3 may offer the opportunity of improving the pathophysiology of metabolic disorders.

P-32-037

Exploring the associations of multifaceted proteins to diseases

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The characterization of multifunctional proteins is an expanding research area aiming to elucidate the complexities of biological processes. We recently developed MultifacetedProtDB (Bertolini et al, 2024, <https://multifacetedprotdb.biocomp.unibo.it>), a curated database providing a collection of 1103 multifunctional human proteins, of which 812 are enzymes. We annotate this collection for disease associations, merging information from UniProt, Humsavar, Monarch, and ClinVar, reporting disease nomenclatures as MONDO, ICD10, OMIM and Orphanet catalogues. Some 30% of proteins in our database (321 enzymes and 110 non-enzymes) are associated with 895 MONDO diseases classified into 213 ICD10 categories and in 17 out of the 19 ICD10 main chapters (excluding chapters without a genetic component). The most represented chapter is XVII: "Congenital malformations, deformations and chromosomal abnormalities" accounting for 226 diseases associated with 135 multifaceted proteins, followed by IV: "Endocrine, nutritional and metabolic diseases", VI: "Diseases of the nervous system", II: "Neoplasm", with 134, 105, and 48 associated multifaceted proteins, respectively. Out of the 895 diseases, 323 are included in the Orphanet catalogue of rare diseases. Over the 431 multifaceted proteins with MONDO disease annotation, 212 are associated with multiple diseases, and 56% are associated also with multiple Reactome pathways. Performing different functions in different pathways could explicate why a protein is associated with different diseases, giving insight to the molecular mechanism leading to disease insurgence. MultifacetedProtDB is useful for characterizing the involvement of a multitasking protein in the cell molecular complexity and associated disease, and possibly future research will enlarge the actual collection and improve its annotation. Bertolini et al. MultifacetedProtDB: a database of human proteins with multiple functions. *Nucleic Acids Res.* 2024 Jan 5;52(D1): D494-D501.