Title

Endothelial Interferon Regulatory Factor 3-activation links inflammation and phenotypic changes through metabolic rewiring

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Research objective

Endothelial cells (ECs) play a crucial role in the progression of both physiological and pathophysiological inflammation. DNA-induced inflammation contributes to the development of a vast array of pathological conditions including cancer, infectious and autoimmune diseases. Currently, extensive research in understanding and modulating disease severity is focused on the STimulator of Interferon Genes (STING) pathway. This study sought to investigate the metabolic and functional consequences of endothelial STING activation.

Methods

Descriptive analysis: qPCR, ELISA, immunoblotting, immunofluorescence, ROS measurements, radioactive metabolite-tracing, metabolomics, seahorse. Functional analysis: scratch migration and spheroid sprouting.

Results

STING-activation alters endothelial phenotype by inducing inflammation and disrupting cell junctions

2'3'-cyclic-GMP-AMP (cGAMP) activates STING-signaling in ECs inducing expression of pro-inflammatory cytokines (TNFa and IL6), chemokines (CXCL9 and CXCL10) and cell-adhesion molecules (VCAM1 and ICAM). STING activation functionally leads to decreased cellular migration and angiogenic capacity of ECs. Investigations of EC morphology after cGAMP-treatment showed that the phenotypic changes might be caused by disruption of vascular endothelial (Ve)-Cadherin junctions and decreased expression of Claudin-5 and Occludin. Cytoskeletal analysis revealed actin rearrangement and decreased lamellipodia formation supporting reduced migratory capacity of ECs. In vivo, STING-activation alters angiogenesis and vessel maturation.

STING-activation increase oxidative phosphorylation without altering glycolysis and fatty acid oxidation

The phenotypic changes induced by cGAMP-treatment were hypothesized to be associated with metabolic rewiring of ECs. Targeted metabolomic analysis of cGAMP-treated ECs revealed lower levels of glycolytic intermediates and amino acids. However, omics analysis showed no change in expression of enzymes involved in glucose or amino acid metabolism. Glycolysis and fatty acid oxidation were also unaltered upon STING-activation. Interestingly, oxidative phosphorylation (OXPHOS)-related transcripts and oxygen consumption rate increased with STING-activation.

OXPHOS inhibition decrease STING-induced inflammation in ECs but fail to rescue junctional rearrangements

Blocking of STING-induced OXPHOS decreased secretion of pro-inflammatory cytokines in vitro. However, STING-induced disruption in Ve-Cadherin junctions was unaltered with OXPHOS inhibition.

STING-induced EC changes depend on Interferon Regulatory Factor (IRF)-3

CRISPR-based KO of STING, IRF3 and type I interferon receptor (IFNAR) in vitro revealed the functional changes induced by STING to be dependent on activation of IRF3 but independent of IFNAR.

Conclusions

STING-activation alters EC morphology by inducing junctional and cytoskeletal rearrangement. The alterations decrease EC capacity to migrate and form vascular sprouts. Mechanistically the alterations depend on IRF3 transcriptional regulation. IRF3-activation results in metabolic rewiring of ECs leading to increased OXPHOS. The rewiring is crucial for induction of EC inflammatory responses but does not control EC morphology.

Founding sources

The study was funded by AIAS-CO-FUND II: GA: MSCA: 754513, Lundbeckfonden: R307-2018-3667, Carlsberg Fonden: CF19-0687, Kræftens Bekæmpelse: R302-A17296; A.P. Møller Fonden: 20-L-0317; Riisfort Fonden and Steno Diabetes Center Aarhus (SDCA).