

Inflammatory activation of human umbilical vein endothelial cells is accompanied by an altered growth factors secretion profile

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Background and Aims

Several studies revealed a significant correlation between chronic inflammation and cancer. Among other reasons, this may be caused by an enhanced secretion of cytokines and growth factors that additionally occur in course of inflammatory activation. These molecules may act as tumor promoters as they influence proliferation, angiogenesis or metastasis of malignant cells. To analyze the impact of endothelial cells within the context of inflammation we used human umbilical vein endothelial cells which were treated alternatively with interleukin-1-beta or tumor necrosis factor alpha as model system.

Methods

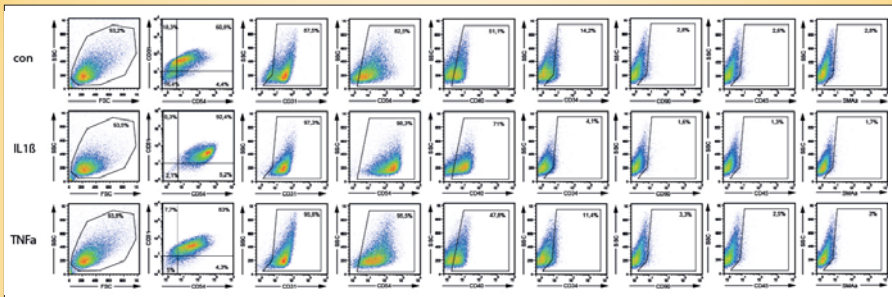
FACS

Flow cytometry analysis (FACS) allows differentiation and quantification of cell types and cell states. The technique is based on the staining of preferentially cell surface antigens of interest (e.g. Cluster of differentiation; CD-antigens) by fluorescence labeled antibodies.

Shotgun proteomics

Untreated and treated cells were fractionated into secreted proteins, cytoplasm and nuclear proteins. These proteins fractions were further separated by SDS PAGE and cut into 6-12 gel slices. Each slice was digested with trypsin and the eluted peptides were analysed by LC-MS/MS (Agilent 1100 system, chip HPLC, Agilent XCT ultra ion trap MS).

Cell characterisation



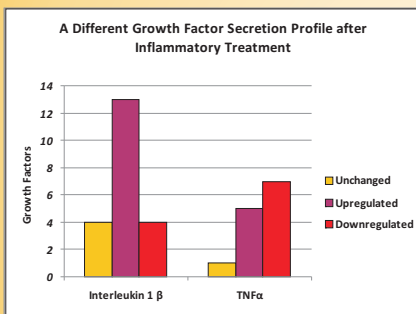
The cells used for proteome profiling were characterised by FACS Analysis with the following antigens:

- SSC (Side Scatter) FSC (Forward Scatter): Determination of homogeneity of cell population and percentage of living cells.
- CD31: Marker for Endothelial cells
- CD40, CD54: Marker to determine the extent of inflammatory activation
- CD90, CD45: Marker for fibroblasts and leukocytes (to determine the absence of contaminating cell types)
- SMAA: smooth muscle actin alpha – Marker for fibrosis

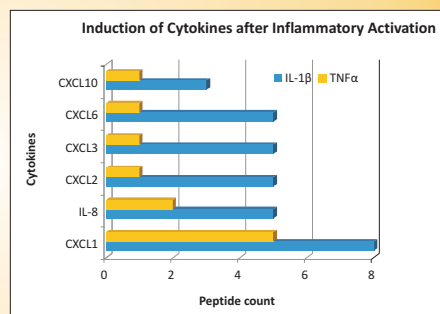
Note that: The inflammatory activation of the homogenous population of ECs was more pronounced in case of IL-1β stimulation compared to TNFα

Results

Secretome profiling was performed by nano LC-MS/MS. We identified a total of 2091 proteins in untreated HUVECs. 192 of them, including 8 growth factors, were genuinely secreted. In IL-1β activated HUVECs we identified a total of 2098 proteins. 201 of those, including 21 growth factors were genuinely secreted. In TNFα activated HUVECs we identified a total of 1875 proteins. 165 of those, including 13 growth factors were genuinely secreted. The number of growth factors quantitatively affected by IL-1β or TNFα treatment is depicted in the following figure.



Endothelial cells were isolated from umbilical veins and treated with IL-1β or TNFα, followed by the analysis of the proteome profile by nano-LC-MS/MS; untreated cells were used as control. Data analysis revealed an altered growth factor secretion profile for stimulated cells in comparison to control.



Endothelial cells were isolated from umbilical veins and treated with IL-1β or TNFα, followed by the analysis of the proteome profile by nano-LC-MS/MS. In case of Interleukin-1-beta stimulation a stronger induction of cytokine production, in comparison to the TNFα dependent response, was observed. The peptide count serves as a semi quantitative measure for protein concentration.

Conclusion

Inflammatory activation of Human umbilical vein endothelial cells either with interleukin-1-beta (IL-1β) or tumor necrosis factor alpha (TNFα) showed an altered growth factor secretion profile. Thereby a stronger induction of growth factors could be observed in case of IL-1β stimulation. Moreover the cytokine secretion profile was found to be dependent on the used stimulus. Similarly to the results observed for the alteration of growth factor secretion IL-1β stimulation resulted in a stronger induction of cytokine production in comparison to the TNFα dependent response. In conclusion the data suggest a possible correlation of the stimulus dependent cytokine induction with an enhanced growth factor secretion.

Systematics

- 1 Bone marrow aspiration of CLL patients In addition isolation of peripheral blood



- 2 Cd19 positive cells isolated by Dynabeads



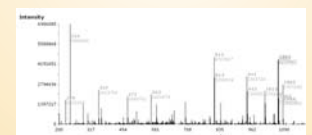
In vitro reference experiments with primary cells derived from healthy donors: treatment with phytohaemagglutinin (PHA-P) and in order to investigate alterations characteristic for proliferation and inflammatory activation

- 3 Isolation of cell nuclei by hypotonic cell lysis and differential centrifugation. Extraction of nuclear proteins by high salt and detergent (NP-40). Protein precipitation to remove non protein impurities and solubilisation in sample buffer.

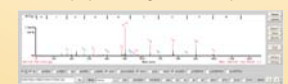
- 4 tryptic digests of gel slices



- 5 nano-LC-MS/MS



- 6 Determination of amino acid sequence out of peptide fragmentation pattern



- 7 Assembly of protein lists

Q5UKD2 mRNA turnover protein 4 homolog - Homo sapiens (Human)
 Q15233 Non-POU domain-containing octamer-binding protein (Klbf2 p)
 Q8WV22 Non-structural maintenance of chromosomes element 1 homo
 Q93863 Nuclear cap-binding protein subunit 1 OS=Homo sapiens GN=4
 P52298 Nuclear cap-binding protein subunit 2 - Homo sapiens (Human)
 Q96A41 Nuclear envelope pore membrane protein POM121 OS=Homo
 Q15F80 Nuclear mitotic apparatus protein 1 (NuMA protein) (SP-Hant g
 P27162 Nuclear pore complex protein Nuq32 OS=Homo sapiens GN=4
 Q9VAM0 Nuclear pore complex protein Nuq33 (Nucleoporin Nuq33) ;
 Q75964 Nuclear pore complex protein Nuq35 - Homo sapiens (Human)
 Q12769 Nuclear pore complex protein Nuq60 OS=Homo sapiens GN=4
 Q97623 Nuclear pore complex protein Nuq205 OS=Homo sapiens GN=4
 P35568 Nuclear pore complex protein Nuq214 (Nucleoporin Nuq214) ;
 Q29567 Nuclear pore complex protein Nuq88 (Nucleoporin Nuq88) (B)
 Q9N177 Nuclear pore complex protein Nuq93 OS=Homo sapiens GN=4
 P22468 Nuclear pore complex protein Nuq94 (Nucleoporin Nuq94)
 P37598 Nuclear pore glycoprotein p62 (62 kDa nucleoporin) - Homo sap
 P82799 Nuclear protein Ncc-1 (Proliferation-associated cyclin-like netes
 Q9N205 Nuclear receptor coactivator 5 OS=Homo sapiens GN=NCAC5 5
 Q9UBJ9 Nuclear RNA export factor 1 (Tip-associating protein) (Tip-asa
 P87909 Nuclear structural element-binding protein 1 (N-bea-binding 2
 Q9BE24 Nuclear GTP-binding protein 1 OS=Homo sapiens GN=GTSP1 4
 Q14978 Nuclear phosphoprotein p130 (Nucleolar 130 kDa protein) (N
 Q92956 Nuclear protein 3 - Homo sapiens (Human)