Introduction.

S.J. Gou, P.C. Xu, M. Chen, M.H. Zhao

vasculitis antibodies in patients with ANCA-associated vasculitis.

Methods.

Six recombinant linear fragments, covering the whole length amino acid sequence of a single chain of MPO, were produced from E. coli. Sera from 77 patients with AAV were presented at a meeting. Thirteen out of the 77 patients had co-existence of serum anti-GBM antibodies. Ten patients also had sequential sera during follow up. The epitope specificities were detected by enzyme-linked immunosorbent assay using the recombinant fragments as solid phase ligands.

Results.

Sera from 45 of the 77 (58.4%) patients with AAV showed a positive reaction to one or more linear fragments of the MPO chain. The Birmingham Vasculitis Activity Scores and the sera creatinine were significantly higher in patients with positive binding to the light chain fragment than that in patients without the binding. The epitopes recognized by MPO-ANCA from patients with co-existence of serum anti-GBM antibodies were mainly located in the N-terminus of the heavy chain. In five out of the six patients, whose sera in relapse recognize linear fragments, the reactivity to linear fragments in relapse was similar to that of initial onset.

Conclusion.

The epitope specificities of MPO-ANCA were associated with disease activity and some clinicopathological features in patients with ANCA-associated vasculitis. The present study may suggest a role of “immunological memory” for inducing relapse in AAV.

Further readings


http://dx.doi.org/10.1016/j.lpm.2013.02.246

P176

Investigating the microRNA signature of ANCA associated vasculitis

N. Brown, S. Harris, M. Venning, P. Brenchley

Manchester Royal Infirmary, Renal Research Labs, Manchester, United Kingdom

Introduction.

MPO positive and PR3 positive ANCA associated vasculitis (AAV) are now established to be distinct genetic and clinical entities. However, the underlying differences in pathogenesis are not fully understood. Epigenetic studies have enabled the discovery of novel pathways involved in disease mechanism and potential therapeutic targets in other diseases. The most abundant epigenetic control mechanism is the production and function of microRNAs (miRs).

We sought to investigate the miR signature of AAV, focusing on differences between MPO and PR3 positive disease.

Methods.

We used pooled plasma from PR3 positive and MPO positive patients in both active and quiescent disease states (total 40 samples) as well as pooled normal plasma. Total RNA was isolated and reverse transcribed to produce cDNA. Real time PCR was performed and results analysed using miScript miRNA Array Data Analysis software. This process was repeated using the TaqMan Array Human microRNA cards for validation of results. The resulting data was examined in silico using mirBase and associated sites to identify predicted gene targets of the differentially expressed miRNAs along with literature review to identify potentially relevant functions of the identified miRs.

Results.

Several differentially expressed miRs were identified in MPO active versus PR3 active disease (validated in comparisons with healthy controls) (table I). The majority of these have roles in T cell regulation.

| Table I |
| --- | --- | --- | --- |
| MicroRNA | Up or down regulated | Gene targets top 10 predicted | Relation to T cell function |
| Hsa-mir-424 Let-7f | Up | FIGN, LINC28, TRIM71, IGDC3, ARGMAP28, ENSSG00000217865, COL1A2, IGK3, CPEB1, BAG1 | Predominantly expressed in naive CD8 T cells.¹ Involved in control of fate of memory T cells.² |
| Hsa-mir-424 | Up | TIBP1, L9Y, PLAG1, FGZ2, CPEB2, RAGFET1B, PAPPA2, SYT4, TLK1, PHP19 | Mir-424 expression has been identified as discriminative of T-lineage versus B-lineage in ALL. Expression increased in patients with TB.³ |

References


Table I (Continued)

<table>
<thead>
<tr>
<th>MicroRNA</th>
<th>Up or down regulated</th>
<th>Gene targets top 10 predicted</th>
<th>Relation to T cell function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsa-mir-106b</td>
<td>Down</td>
<td>ZNF51, TNKS2, SERF1A, SERF1B, PKD2C270F245, V5X1, PLXRA3, ITG68, EZF5</td>
<td>Mir-106b has been shown to modulate the TGF-signaling pathway (with TGF-involved in T regulatory cells differentiation and maturation).</td>
</tr>
<tr>
<td>Hsa-mir-9</td>
<td>Down</td>
<td>ONECUT2, TEKX2, CSDA, RNF111, COL15A1, POL2F1, PRDM6, BEND3, UBE3C, MUM1L1</td>
<td>Mir-9 enhances IL-2 production in activated human CD4+ T cells by repressing Blimp-1.</td>
</tr>
<tr>
<td>Hsa-mir-125a</td>
<td>Down</td>
<td>PCTP, TRIM71, TMEM477, ENPP1, SMEK1, XAA152, ENPD2, RNF543, MFHAS1, ADAM9</td>
<td>MicroRNA-125a negatively regulates RANTES expression by targeting KLF13 in activated T cells.</td>
</tr>
<tr>
<td>Hsa-mir-15b</td>
<td>Down</td>
<td>CPEB2, CCN1, FGF2, PIAG1, R5P03, STX2, WEE1, ZBTB34, SLC20A2, MGAT4A</td>
<td>Predominantly expressed in memory T cells (one of 7 mRNAs constituting 60% of the mRNAs expressed in CD8+ T cells).</td>
</tr>
</tbody>
</table>

Discussion.— This novel preliminary data generates the hypothesis that further investigation of miR profiles may help explain underlying differences in immune pathogenesis between MPO and PR3 positive disease. Conclusion.— Further validation of these results in a larger population is required to establish the potential significance and function of these differences in miR expression. This may allow future use of microRNAs in AAV as biomarkers or to enable identification of novel therapeutic targets.

Further readings

http://dx.doi.org/10.1016/j.jlpm.2013.02.247

P177
The interaction between CSa and sphingosine-1-phosphate in neutrophils for ANCA-mediated activation
J. Hao, C. Wang, M. Chen, M.H. Zhao Peking University First Hospital, Beijing, China

Introduction.— CSa and the neutrophil CSa receptor play a central role in antineutrophil cytoplasmic antibody (ANCA)-mediated neutrophil recruitment and activation. The current study further investigated the interaction between CSa and S1P in neutrophils for ANCA-mediated activation. Methods.— The levels of S1P in plasma sequential samples from 29 patients with ANCA-associated vasculitis (AAV) in active stage and in remission were tested. The effect S1PR inhibitor was tested on respiratory burst and degranulation of CSa-primed neutrophils activated with ANCA. Results.— The level of S1P was significantly higher in active disease than that in remission (2034.2 ± 438.5 vs. 1489.3 ± 547.4, P < 0.001). S1P-primed neutrophils activated with MPO-ANCA-positive IgG and PR3-ANCA-positive IgG (314.0 ± 36.8 vs. 379.0 ± 20.6, P < 0.05; 314.0 ± 36.8 vs. 384.5 ± 11.4, P < 0.05). S1P-primed neutrophils induced by MPO or PR3-ANCA-positive IgG, the lactoferrin concentration in the supernatant increased from 448.0 ± 17.1 ng/ml in untreated cells to 1342.7 ± 29.5 ng/ml (P < 0.001) or 1338.3 ± 27.1 ng/ml (P < 0.001). The level of S1P increased from 18.0 ± 3.0 nmol/L in the non-primed neutrophils supernatant to 53.3 ± 4.2 nmol/L in CSa-primed neutrophils supernatant (P < 0.01), and decreased to 24.7 ± 3.2 nmol/L upon pre-incubation with CSaR (CD88) inhibitor (P < 0.05). In CSa-primed neutrophils, subsequently activating with MPO or PR3-ANCA-positive IgG, the MFI value was 369.8 ± 18.8 (P < 0.01) or 377.3 ± 13.2 (P < 0.01), which decreased to 310.0 ± 12.5 (P < 0.01) or 323.8 ± 15.5 (P < 0.05) upon pre-incubation with S1PR inhibitor. Conclusion.— The level of S1P is significantly higher in active AAV patients than that in remission. S1P triggered by CSa-primed neutrophils acting as an autocrine or paracrine manner, which activate neutrophils again. Blocking S1PR may decrease CSa-induced activation of neutrophils by ANCA. The interaction between S1P and CSa may play an important role in neutrophils for ANCA-mediated activation.

http://dx.doi.org/10.1016/j.jlpm.2013.02.248

P178
Circulating level of high mobility group box-1 is associated with disease activity in antineutrophil cytoplasmic autoantibody-associated vasculitis
C. Wang, M. Chen Renal Division, Peking University First Hospital, Beijing, China

Introduction.— High mobility group box-1 (HMGB1) is a kind of pro-inflammatory mediator and has been confirmed to be associated with inflammatory conditions and tissue damage. Previous studies have reported circulating HMGB1 levels were elevated in patients with active ANCA-associated vasculitis (AAV). The current study aimed to...