Fli-1, a master regulator in modulating expression of inflammatory mediators in endothelial cells

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THE HUMAN ETS GENE FAMILY

Pointed Domain

ETS1
ETS2
ERG2
ELK1
SPI1 (PU.1)
FLI1 (ERGB)
SAP1 (ELK4)
ELF1
SPIB
E4TF1 (GABP)
E1AF (PEA3/ETV4)
PE1 (ETV3)
ERM (ETV5)
TEL (ETV6)
NET (SAP2/ERP/ELK3)
ERF
ETV1 (ER81)
NERF2
MEF
ESX (JEN/ESE/ERT/ELF3)
FEV
EHF (ESE3)
ELF5 (ESE3)
PDEF (ESF/PSE)
TREF (TEL2)

ETS Domain

100 a/a
Fli–1 Transcription Factor

- Two transcriptional activation domains: amino-terminal transactivation domain (ATA) and carboxy-terminal transactivation domain (CTA)
- Removal of CTA domain of Fli–1 reduces \textit{in vitro} transcriptional activation activity by 40–50%

DIAGRAM:
- **PNT** (115-195)
- **ATA** (106-271)
- **ETS Domain** (277-361)
- **CTA** (402-452)
• Glomerulonephritis is a major cause of death in both human and animal models.

• The incidence of renal involvement in lupus patients is from 25-75% depending on the reports. The general consensus is that 60% of lupus patients will develop lupus nephritis at some time.

Overexpression (2-3 fold) of the Fli-1 protein in transgenic mice results in the development of a lupus-like disease, including:

* Autoantibody production including anti-DNA
* Renal deposition of immune complexes
* Hypergammaglobulinemia
* Increased autoreactive T and B cells
* Death due to glomerulonephritis

Fli-1 Gene and lupus autoimmune disease development

• Overexpression of Fli-1 mRNA was found in lymphocytes from active lupus patients compared to normals.

• NZB/NZW mouse splenocytes have higher expression levels of Fli-1 mRNA compared to BALB/c mice.

Decreased expression of Fli-1 in Fli-1+/- NZM2410 mice
Ninety-three percent of Fli-1\(^{+/-}\) NZM2410 mice (n=14) survived to age 52 weeks compared with only 35% of Fli-1\(^{+/+}\) NZM2410 mice (n=23)
Mice were sacrificed at the age of 34 weeks. The kidneys were removed from WT (n=21) and Fli-1+/- (n=18).

<table>
<thead>
<tr>
<th>Genotypes of mice</th>
<th>Infiltrated T cells (10 HPF*)</th>
<th>Infiltrated neutrophil (10 HPF)</th>
<th>Infiltrated macrophages (10 HPF)</th>
<th>Infiltrated B cells (10 HPF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>44.00 ± 7.6</td>
<td>19.57 ± 2.5</td>
<td>58.61±10.5</td>
<td>33.59±5.6</td>
</tr>
<tr>
<td>Fli-1+/-</td>
<td>21.81 ± 6.0</td>
<td>11.00 ± 1.6</td>
<td>20.18±4.6</td>
<td>8.7±2.8</td>
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<tr>
<td></td>
<td>( P&lt;0.01 )</td>
<td>( P&lt;0.01 )</td>
<td>( P&lt;0.01 )</td>
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</tbody>
</table>
TLRs are critical to the function of the innate immune response
- TLRs recognize pathogen-associated molecular patterns.
- Trigger a strong, sudden immune response
- Expressed on a variety of cells, including immune cells.
- 13 different types of TLRs have been identified in mouse, each recognizing a unique pattern.
Chemokine (C-X-C motif) ligand 2 (CXCL2)

- Chemokine (C-X-C motif) ligand 2 (CXCL2) is a small cytokine (107 amino acids) belonging to the CXC chemokine family that is also called macrophage inflammatory protein 2-alpha (MIP2-alpha), Growth-regulated protein beta (Gro-beta) and Gro oncogene-2 (Gro-2).

- CXCL2 is chemotactic for polymorphonuclear leukocytes and hematopoietic stem cells.

- CXCL2 is 90% identical in amino acid sequence as a related chemokine, CXCL1.

- It has been reported that expression of CXCL2 is regulated by NFkB.
Fli-1 regulates expression of MCP-1, IL-6, and CCL5


Decreased expression of Fli-1 in endothelial cells after transfected with Fli-1 siRNA
Inhibition of Fli-1 resulted in decreased production of CXCL2 in endothelial cells after TLR4 stimulation.
Production of CXCL2 was increased by dose-dependent LPS stimulation
Inhibition of Fli-1 resulted in decreased production of CXCL2 in endothelial cells after TNF-α stimulation.
Fli-1 drives transcription from the CXCL2 promoter
Increased expression of Fli-1 protein with higher amount of plasmid transfected
Fli-1 Binds to the promoter of CXCL2
Structure of Fli-1 Protein

- PNT: 115-195
- ETS Domain: 277-361
- ATA: 106-271
- CTA: 402-452
Fli-1 regulates CXCL2 through direct binding of the promoter.
Inhibition of Fli-1 resulted in decreased production of CXCL2 in human umbilical cord endothelial cells after TLR4 stimulation.
The endothelium is composed of $1-6 \times 10^{13}$ endothelial cells lining a total area of 7000 $M^2$.

Endothelial cells play an important role in the trafficking of immune cells, as well as inflammation.

A limited research on the role of endothelial cells in response to inflammatory stimulation.
Football is played on a field: 360 by 160 feet (120.0 by 53.3 yards; 109.7 by 48.8 meters.)

\[109.7 \times 48.8 = 5353.36 \text{M}^2\]

\[7000/5353.36 = 1.3\]
Fli-1 transcription factor is novel regulator in modulating inflammatory mediators
Lab members:
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Sarah Williams
Jeremy Mathenia
Eva Karam
Mara Lennard-Richard
Shuzo Sato
Emmanuel Ryes-Cortes
Ivan Molano
Danielle Brandon
Nicole Sztokman
Ning Lou

Collaborators:
Tammy Nowling
Dennis Watson
Gary Gilkeson

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Summary

- Fli-1 is a master regulator in modulating expression of inflammatory mediators in endothelial cells.

- One of the mechanisms that Fli-1 impacts lupus disease is to regulate expression of important inflammatory mediators.

- Manipulating expression of Fli-1 may have therapeutic effects on many inflammatory diseases.
Cytokines, Chemokines

Cytokines: a broad and loose category of small proteins (~5–20 kDa) that are important in cell signaling. They are released by cells and affect the behavior of other cells, and sometimes the releasing cell itself.

Chemokines: a family of small cytokines, or proteins secreted by cells. Their name is derived from their ability to induce directed chemotaxis in nearby responsive cells; they are chemotactic cytokines.
Structure of Chemokines

Structure of chemokine classes

C chemokines

CC chemokines

CXC chemokines

CX3C chemokines

peptide chain

disulphide bridge

hydrophobe domain

mucine-like domain
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