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Regulation of the beta7 integrin gene in T cells-
Role of the MAPK signalling pathways

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A Thesis submitted for the degree of Doctor of Philosophy at The University of Auckland, Auckland, New Zealand

October 2004
To my dear parents, for without their love and support, this would not have been possible.
Abstract

Members of the integrin superfamily of adhesion molecules are involved in cell to cell, cell to ECM, and cell to pathogen interactions, and are of fundamental importance in many biological processes. The β7 integrins α4β7 and αEβ7 have evolved to play specialized roles in gut mucosal immunity. α4β7 mediates the homing of lymphocytes to intestinal Peyer’s patches, mesenteric lymph nodes, and the lamina propria by binding to the vascular addressin MAdCAM-1 (Fong et al., 1997). αEβ7 binds epithelial E-cadherin retaining lymphocytes at the intraepithelial compartment of the mucosa. The expression of αEβ7 is induced on migratory lymphocytes by TGF-β secreted by gut enterocytes. The signalling mechanisms responsible for basal and TGF-β-induced expression of β7 integrins are not well understood. Previous studies identified two TGF-β1 response regions in the β7 gene promoter termed TGFBRRI and TGFBRRII encompassing nucleotides -509 to -398 and -121 to -34. Here, TGF-β1 activation of the β7 gene proximal promoter is shown to be mediated by MAPK family members. TGF-β1 stimulation of TK-1 T cells increased the steady-state mRNA levels of the β7 gene relative to α4 transcripts, and led to enhanced phosphorylation of c-Jun. TGF-β1 stimulation induced rapid increases in the binding of nuclear protein complexes to TGFBRRI and -2. Sp1 and Sp3 which mediate TGF-β1 signalling were shown to bind to an Sp1 cis-element encompassing nucleotide positions -67 to -60 within TGFBRRII. The interaction of Sp1 with its cognate binding site was c-Jun dependent. In contrast, there was no evidence for involvement of the Smad proteins. ATF-2 was identified to bind to a region encompassing nucleotide positions -699 to -689 just upstream of TGFBRRI. Sp1 and ATF-2 expression vectors co-transfected into Sp1-deficient SL-2 cells synergized to drive the activity of a β7 gene luciferase reporter. Mutation of the ATF-2-binding site modestly reduced β7 gene reporter activity.
The involvement of c-Jun in TGF-β signalling and interaction of Sp1 with the β7 gene promoter suggested that MAP kinase pathways might mediate β7 gene transcription. Specific chemical inhibitors were used to ascertain which of the three MAPK pathways namely p38, JNK, and ERK were involved. Results obtained by nuclear run-on transcription analysis which measures nascent RNA synthesis showed that both the p38 and JNK pathways regulate β7 gene expression in TK-1 cells, whereas only the p38 pathway regulates α4 gene expression. Thus, treatment of TK-1 cells with the p38 inhibitor SB203580 and the JNK inhibitor SP600125 inhibited the synthesis of β7 transcripts, whereas only SB203580 inhibited the synthesis of α4 transcripts. Conversely, sodium arsenite which induces JNK and p38 upregulated nascent synthesis of α4 and β7 RNA transcripts. SB203580 blocked the binding of nuclear factors to TGFBR-1, and ATF-2 binding to nucleotide position -699 to -689. Similarly, SP600125 blocked the binding of Sp1 and Sp3 to TGFBR-2, whereas unexpectedly SB203580 enhanced their binding. Furthermore, both SB203580 and SP600125 decreased cell-surface expression of the β7 subunit and SB203580 inhibited TK-1 cell adhesion to MAdCAM-1. In contrast, the MEK inhibitor PD98059 had no effect on the expression of nascent β7 RNA transcripts and cell-surface expression of the β7 subunit, suggesting that the ERK pathway is not involved in regulation of β7 gene expression in TK-1 cells. In contrast to the results obtained with TK-1 cells, SB203580, SP600125, and PD98059 each inhibited the nascent synthesis of α4, β7, and αE transcripts in peripheral blood lymphocytes.

In conclusion, this study has revealed for the first time that both the p38 and JNK pathways mediate TGF-β1-induced expression of the integrin β7 gene. Expression of the β7 gene is Sp1-dependent, and involves the synergistic cooperation of c-Jun and ATF-2. It is proposed that the p38 and JNK pathways play a role by triggering the activation and translocation of c-Jun and ATF-2 to the nucleus.
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First and foremost, I would like to express my gratitude to my supervisor Associate Professor Geoffrey Krissansen for all his guidance, advice, and support throughout the course of this study. He has been a great mentor, and his patience, positive attitude, and work ethic have taught me a great deal at a personal level. I would also like to thank my advisor Dr Euphemia Leung who has a wealth of technical knowledge in the field and my colleague Mr Sushil Pandey for many helpful discussions and the opportunity to present two relevant results from his work namely Figures 3.6 and 3.8 in this thesis. I would like to thank Ms Yi Yang for preparation of recombinant cell adhesion molecules and help with the FACS analysis. My gratitude also extends to other laboratory colleagues including Dr Lidija Petreska, Dr Klaus Lehnert, Dr Jagat Kanwar, Dr Rupinder Kanwar, Dr Ji Zhong Bai, Mr Yih Chih Chan, and Mrs Rita Gupta for many helpful discussions and suggestions.

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Abbreviations

A absorbance
Act D actinomycin D
ATP adenosine triphosphate
AMP’ ampicillin resistance
ATCC American Type Culture Collection
bp base pair
cDNA complementary DNA
Ci curies
© copyright
dATP deoxyadenosine triphosphate
dCTP deoxycytidine triphosphate
dGTP deoxyguanosine triphosphate
dNTP deoxynucleoside triphosphate
dTTP deoxythymidine triphosphate
DEPC diethylpyrocarbonate
DMSO dimethylsulfoxide
DNA deoxyribonucleic acid
DTT dithiothreitol
ECM extracellular matrix
EDTA ethylenediaminetetra-acetic acid
ERK extracellular signal-regulated protein kinase
EtBr ethidium bromide
FACS fluorescence-activated cell sorter
FMLP f-methionine-leucine-phenylalanine
FN fibronectin
fpu foot print unit
G3 glyceraldehyde-3-phosphate dehydrogenase
h hour
HBS hepes-buffered saline
HEV high endothelial venules
HBSS Hanks balanced salt solution
ICAM-1 intercellular adhesion molecule-1
IFN-γ interferon-γ
IEL intraepithelial lymphocytes
IL interleukin
IMAGE integrated molecular analysis of genomes and their expression
JNK c-Jun N-terminal kinase
kb kilobase
kDa kilodalton
<table>
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<th>Abbreviation</th>
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<tr>
<td>TAK1</td>
<td>TGF-β activated kinase 1</td>
</tr>
<tr>
<td>TBE</td>
<td>tris-borate-EDTA</td>
</tr>
<tr>
<td>TEMED</td>
<td>tetramethylethylenediamine</td>
</tr>
<tr>
<td>TfbI</td>
<td>transformation buffer I</td>
</tr>
<tr>
<td>TfbII</td>
<td>transformation buffer II</td>
</tr>
<tr>
<td>TGF-β</td>
<td>transforming growth factor-β</td>
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<td>tumour necrosis factor-α</td>
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