Expression of the
uncharacterised isoform, BCL2β,
in melanoma

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Statement of Originality

The thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University’s Digital Repository, subject to the provisions of the Copyright Act 1968.

Signed,

Chloe Warren
Acknowledgements and Dedications

I’d like to thank my wonderful and supportive supervisor, Dr Nikola Bowden, without whom I would have mistaken my first stumble for what it felt like at the time (i.e. a bear trap), and abandoned this journey pretty much immediately. You are the greatest mentor I could have hoped for and I will never forget your kindness, compassion, patience and wisdom.

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# Abbreviations

<table>
<thead>
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<th>ABBREVIATION</th>
<th>MEANING</th>
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<tr>
<td>ALM</td>
<td>acral lentiginous melanoma</td>
</tr>
<tr>
<td>AMO</td>
<td>antisense morpholino oligonucleotides</td>
</tr>
<tr>
<td>BER</td>
<td>base excision repair</td>
</tr>
<tr>
<td>BH</td>
<td>BCL2 homology</td>
</tr>
<tr>
<td>BRAF V600E</td>
<td>T to A substitution at codon 600 in the BRAF gene</td>
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<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
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<td>CLL</td>
<td>chronic lymphoid leukaemia</td>
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<tr>
<td>CPD</td>
<td>cyclopyramidine dimers</td>
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<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagle Medium</td>
</tr>
<tr>
<td>DSB</td>
<td>double strand break</td>
</tr>
<tr>
<td>dsDNA</td>
<td>double stranded DNA</td>
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<td>DTIC</td>
<td>dacarbazine</td>
</tr>
<tr>
<td>ER</td>
<td>endoplasmic reticulum</td>
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<tr>
<td>ESI</td>
<td>electron spray ionisation</td>
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<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
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<tr>
<td>IHC</td>
<td>immunohistochemistry</td>
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<tr>
<td>IP</td>
<td>immunoprecipitation</td>
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<tr>
<td>LMM</td>
<td>Lentigo maligna melanoma</td>
</tr>
<tr>
<td>LSB</td>
<td>Antibody LS-B11858 (Life Span Bioscience)</td>
</tr>
<tr>
<td>m/z</td>
<td>mass/charge</td>
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<tr>
<td>MMR</td>
<td>mismatch repair</td>
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<tr>
<td>MOM</td>
<td>mitochondrial outer membrane</td>
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<tr>
<td>MOMP</td>
<td>mitochondrial outer membrane permeability</td>
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<tr>
<td>Acronym</td>
<td>Full Form</td>
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<td>MRM</td>
<td>multiple reaction monitoring</td>
</tr>
<tr>
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<td>O/N</td>
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<td>transcription factor</td>
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<td>Australian Therapeutic Goods Administration</td>
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<td>TIL</td>
<td>tumour infiltrating lymphocytes</td>
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</tr>
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<td>well plate</td>
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<td>voltage dependent ion channel</td>
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Abstract

There are two known isoforms of the anti-apoptotic protein BCL2. While BCL2α is well characterised and known predominantly for its role in apoptosis, BCL2β has not yet been described. We sought to confirm the presence of this isoform in melanoma and characterise its role in the apoptotic response.

We were able to verify expression of the rare isoform at the protein level in cell lines using multiple reaction monitoring tandem mass spectrometry. We also examined the role of the isoforms in apoptosis and melanin synthesis by a) monitoring gene response to stress at the mRNA level and b) using siRNAs targetted to each individual transcript. We treated cells with a variety of stressors and then monitored their apoptotic response using flow cytometry. We also quantified melanin production in response to UVB.

The BCL2α response to stress (i.e. downregulation prior to apoptosis) was matched across melanocyte and melanoma cell lines. However, BCL2β response was varied across the melanoma cell lines, but matched that of BCL2α in melanocytes. Knock-down of both isoforms (individually) resulted in increased apoptosis in melanoma cell lines.

We quantified mRNA of both BCL2 isoforms in our cohort of 189 FFPE melanoma tumours using qPCR. We also quantified total BCL2 protein expression in this same cohort using immunohistochemistry.

Interestingly, expression of the BCL2β isoform in tumours was significantly associated with increased overall survival (686.4 weeks, 95% CI 462.5-910.3). BCL2β expression was also elevated in metastatic tissue compared with
primary. This pattern was actually seen in the reverse at the protein level; total BCL2 protein was elevated in primary compared with metastatic.

Analysis of SNP rs3943258 revealed that possession of the mutant T allele in melanoma patients corresponded with increased BCL2β expression. This contrasts with previous observations from a different study on healthy controls.

Our current understanding of the role of BCL2β is based on the concept that it lacks the C-terminal transmembrane domain, and is thus incapable of localising to target organelles. It is generally assumed that the isoform is of null function. However, these observations have been based based on studies of non-representative synthetic versions of BCL2β. This is the first time the naturally transcribed version of the rare isoform has been studied, and the first time its role has been investigated since the 1980s. We have demonstrated herein that BCL2β performs an anti-apoptotic role within the cell, and that its regulation may be disrupted in melanoma.