The International Journal of Biochemistry & Cell Biology 59 (2015) 94–102

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Role of the pro-survival molecule Bfl-1 in melanoma

C.K. Hind, M.J. Carter, C.L. Harris, H.T.C. Chan, S. James, M.S. Cragg*

Antibody and Vaccine Group, Cancer Sciences Unit, University of Southampton, Faculty of Medicine, Southampton General Hospital, Southampton SO16 6YD, UK

ARTICLE INFO

Article history:
Received 9 September 2014
Received in revised form
11 November 2014
Accepted 28 November 2014
Available online 6 December 2014

Keywords:
Bfl-1
BCL2A1
Apoptosis
Melanoma
Melanocytes

ABSTRACT

Bfl-1 is a pro-survival Bcl-2 family member overexpressed in a subset of chemoresistant tumours, including melanoma. Here, we characterised the expression and regulation of Bfl-1 in normal and malignant melanocytes and determined its role in protecting these cells from chemotherapy-induced apoptosis. Bfl-1 was mitochondrially resident in both resting and apoptotic cells and experienced regulation by the proteasome and NFκB pathways. siRNA-mediated knockdown enhanced sensitivity towards various relevant drug treatments, with forced overexpression of Bfl-1 protective. These findings identify Bfl-1 as a contributor towards therapeutic resistance in melanoma cells and support the use of NFκB inhibitors alongside current treatment strategies.

1. Introduction

Metastatic melanoma is an aggressive form of skin cancer with only a 20% 5 year survival rate when diagnosed at late stages (Buzzell and Zitelli, 1996). Increases in its incidence, attributable to an ageing population and the increased use of sunbeds, combine with extensive resistance to conventional chemotherapeutics to provide an increasingly problematic cancer in the developed world (Wang et al., 2001; Soengas and Lowe, 2003; Walter et al., 1992). Such chemoresistance can be achieved through dysregulation of apoptosis, commonly via modulation of Bcl-2 family member expression and function (Soengas and Lowe, 2003).

The Bcl-2 family regulates multiple forms of cell death with a central role in apoptosis (Chipuk et al., 2010; Vogler, 2012). It is subdivided into two distinct groups: the pro-survival members (Bcl-2, Bcl-X, Bcl-w, Mcl-1, Bfl-1) and the pro-apoptotic members, which are further subdivided into the multidomain Bax/Bak-like proteins (Bax, Bak, Bok) and the BH3-only proteins (Bim, Bid, Bik, Bmf, Puma, Noxa, Hrk, Bad) (Huang and Strasser, 2000). Whilst there remains debate as to the exact mechanism, the Bcl-2 family appears to regulate mitochondrial outer membrane permeabilisation (MOMP), linking stress stimuli to downstream apoptotic effectors.

The Bcl-2 family directly influence cellular survival and, consequently, are often dysregulated in cancers (Youle and Strasser, 2008). As such, increased expression of pro-survival members such as Bcl-2 can facilitate evasion of apoptosis, one of the hallmarks of cancer (Hanahan and Weinberg, 2011). Microarray data obtained from the National Cancer Institute's panel of 60 cancer-derived cell-lines (NCI60) demonstrates differential expression of pro-survival Bcl-2 family members between tumour types (Fig. 1). Whilst Bcl-2 and Mcl-1 have a broad expression pattern, Bfl-1 was highly and specifically expressed in cell-lines derived from melanoma. High Bfl-1 expression has previously been observed in a selection of other chemoresistant malignancies (Nagy et al., 2003; Piva et al., 2006; Riker et al., 2008) and associated with resistance to apoptosis in CLL (Morales et al., 2005). Taken together, such observations indicate that Bfl-1 may contribute towards the chemoresistance observed in melanoma.

Bfl-1 is a 175-residue protein encoded by the BCL2A1 gene located on chromosome 15q24.3 (Choi et al., 1997). Its subcellular localisation appears cell-specific, with studies suggesting mitochondrial (Duriez et al., 2000) or cytoplasmic (Orlofsky et al., 1999) residency with apoptotic stimuli triggering translocation to the nucleus (Orlofsky et al., 1999). Such multi-compartment residency is reminiscent of that displayed by Bax (Wolter et al., 1997). Consequently, it has been suggested that Bfl-1 subcellular localisation is dictated by the exposure of the C-terminal α9 helix by anchoring to the mitochondrial outer membrane (Brien et al., 2009). Studies concerning Bfl-1 subcellular localisation, however, have been hampered by the observation that GFP-tagging

* Corresponding author. Tel.: +44 2381208452; fax: +44 0 23 80704061.
E-mail address: msc@soton.ac.uk (M.S. Cragg).

http://dx.doi.org/10.1016/j.biocel.2014.11.015
1357-2725/

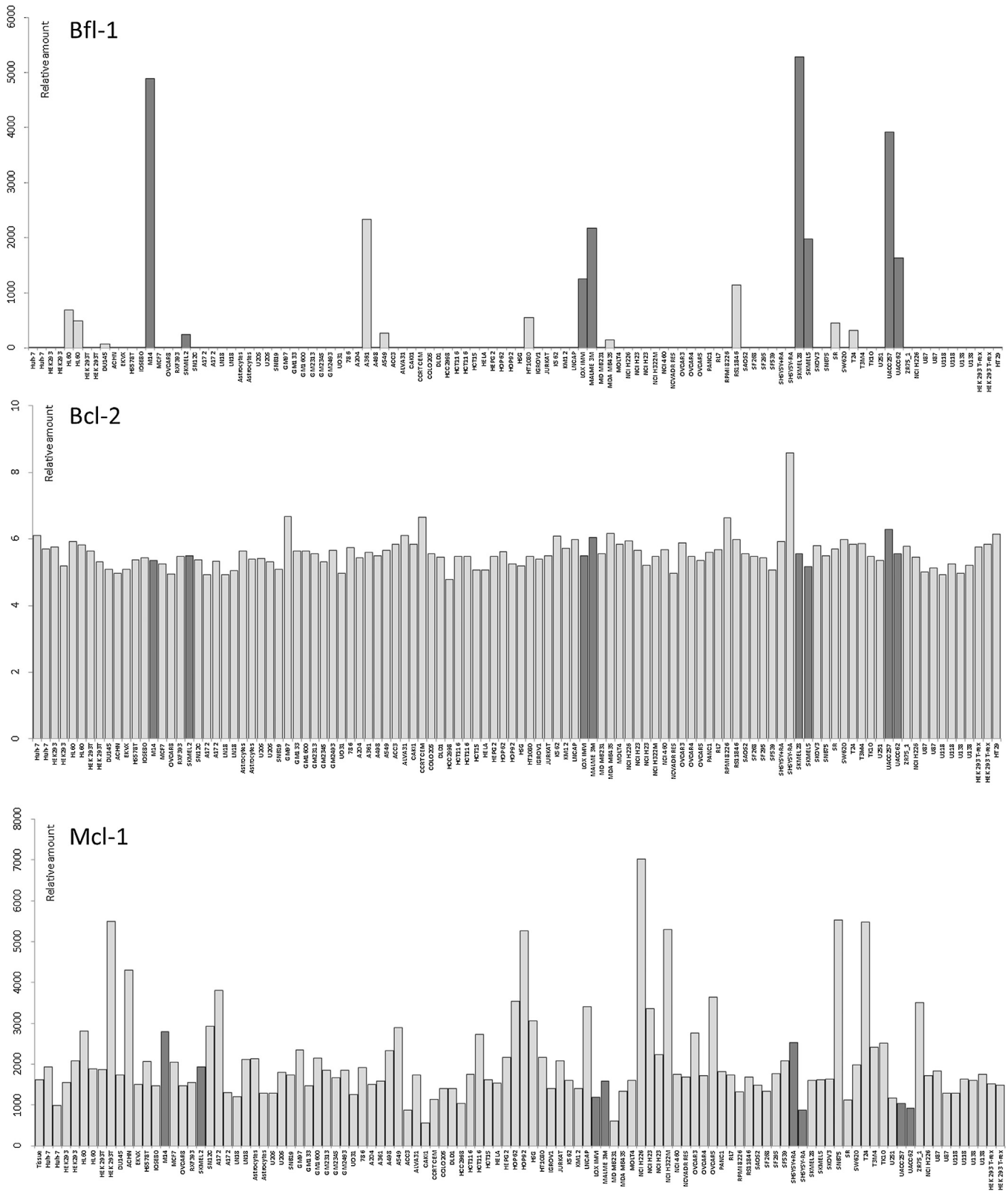

Fig. 1. mRNA expression profiling of Bfl-1, Bcl-2 and Mcl-1 on NCI60 cells. Data taken from http://biogps.gnf.org/. Darker bars on the microarray bar graph indicate the cell-lines derived from melanoma.

may convert Bfl-1 into a pro-apoptotic protein in some cells (Yang et al., 2005).

In addition to the full-length Bfl-1, a truncated splice variant has also been observed in humans, Bfl-1 short (Bfl-1s). Bfl-1s lacks 12 amino-acids in the C-terminus, resulting in localisation to the nucleus (Ko et al., 2003). Bfl-1s originates via alternate mRNA splicing, resulting in incorporation of an additional 56 bp exon (exon II) which creates a frame shift resulting in premature translational termination and production of the 163 residue Bfl-1s (Ko et al., 2003). Although widely accepted, Bfl-1s has been under-studied