Paracrine communication by Bcl-2 overexpressing melanoma cells promotes differentiation and recruitment of macrophages

24

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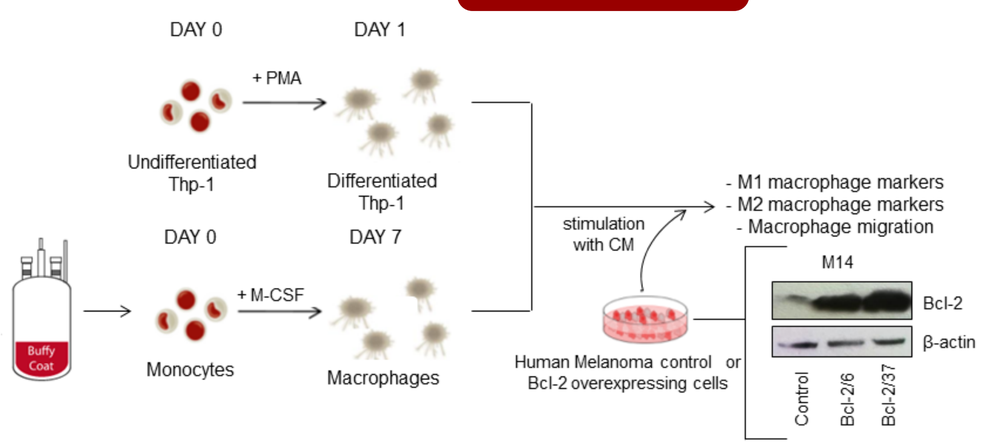
Background

Cutaneous melanoma is an highly aggressive cancer with metastatic behavior. Being a relevant constituent of the tumor microenvironment, tumor-associated macrophages may regulate melanoma progression, through distinct pro-inflammatory (M1) vs pro-tumor (M2) polarized programs. Our previous studies have demonstrated that melanoma overexpressing bcl-2, one of the most crucial regulators of cell apoptosis, show an increased progression, metastatization and vascularization.

Aim

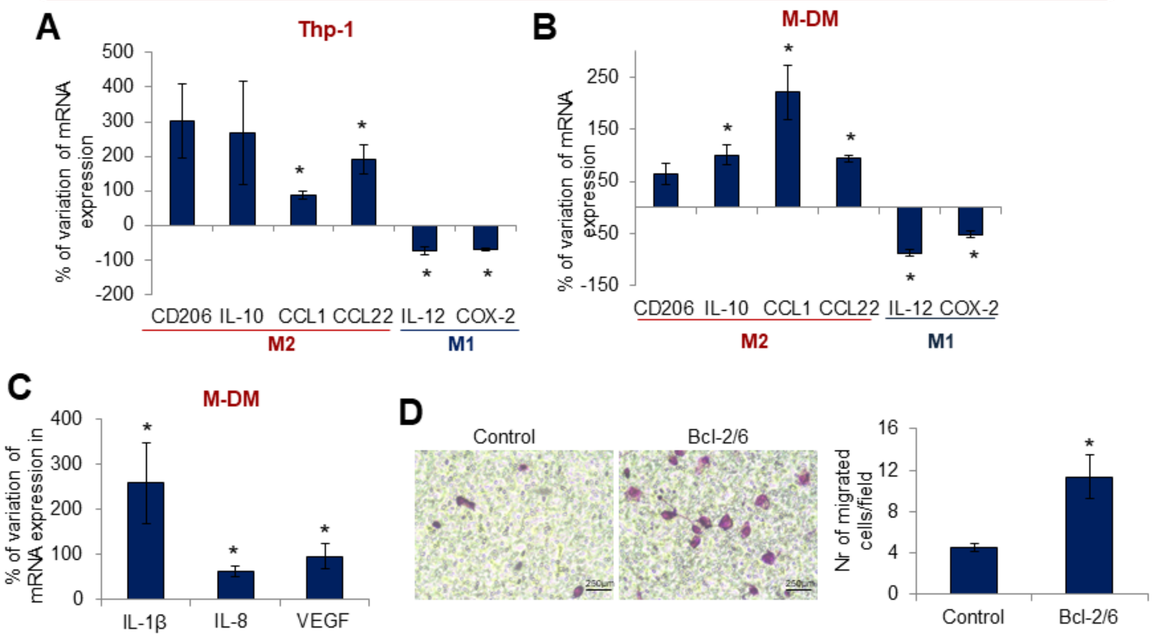
To evaluate whether bcl-2 overexpression in melanoma cells might influence tumor-promoting and polarized functions of tumor-associated macrophages.

Methods



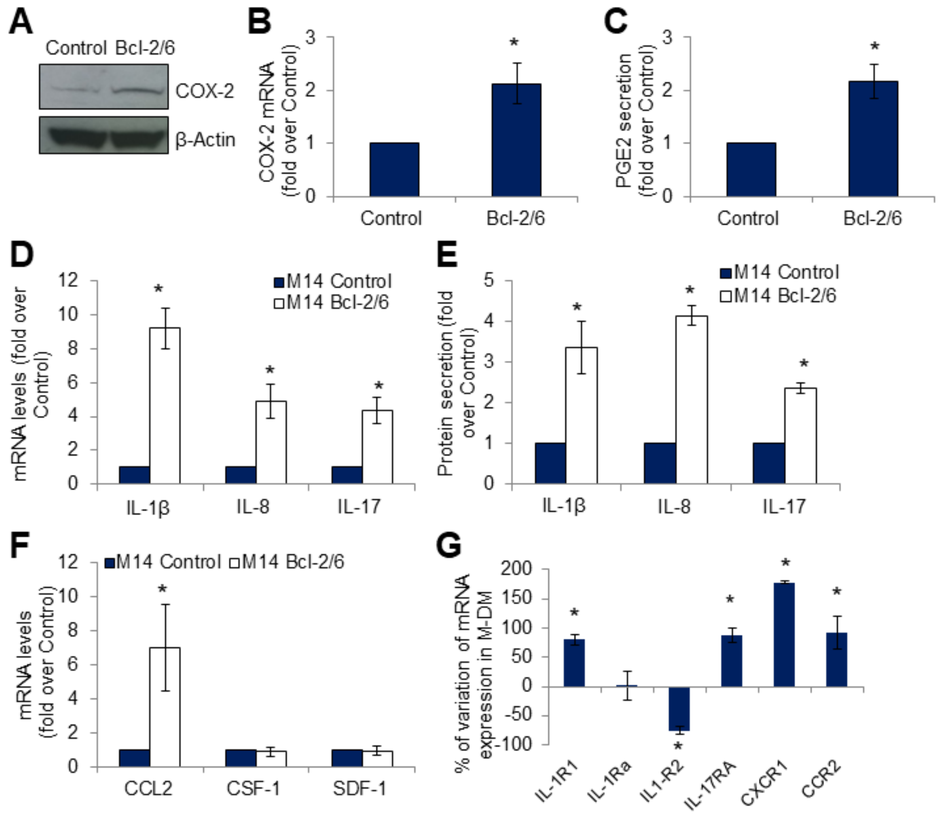
Monocytes isolated from healthy donors' buffy coats were treated with macrophage colony-stimulating factor (M-CSF) for 7 days to differentiate into mature Monocytes-Derived Macrophages (M-DM). Thp1 leukemic monocytic cells were treated with PMA for 24h to differentiate into mature macrophages. M-DM and Thp-1 were exposed to Conditioned Media (CM) obtained from control or bcl-2 overexpressing M14 melanoma cells and their effect on M1 and M2 markers, as well as on macrophage migration were assessed.

Bcl-2 overexpressing melanoma cells promote macrophage migration and polarization towards an M2-like phenotype



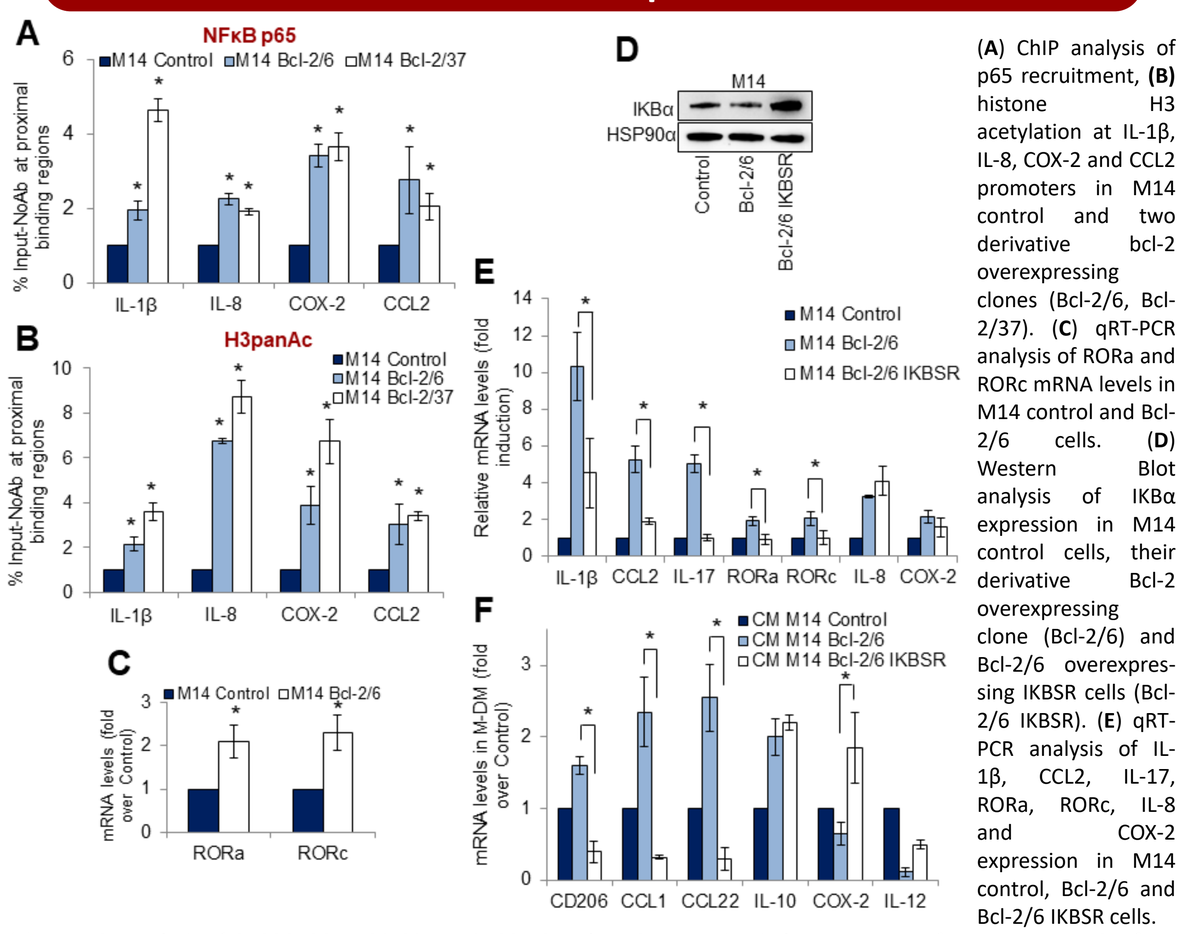
qRT-PCR analysis of CD206, IL-10, CCL1, CCL22, IL-12, COX-2 mRNA levels in (**A**) Thp1 and (**B**) human monocyte-derived macrophages (M-DM) after 24h exposure to CM derived from M14 control or bcl-2 overexpressing cells. **(C)** qRT-PCR of IL-1β, IL-8 and VEGF mRNA levels in M-DM stimulated as reported in **(B)**. **(D)** Representative images and relative quantification of Thp-1 cells migration in response to CM derived from M14 control (Control) or bcl-2 overexpressing (Bcl-2/6) melanoma cells. **(A-C)** Results are reported as % mRNA variation in macrophages exposed to CM derived from Bcl-2/6 versus control one **(A-D)** *p<0.05.

Bcl-2 overexpressing melanoma cells increase the expression of selected inflammatory molecules



(A) Western Blot and **(B)** qRT-PCR analyses of COX-2 expression in M14 control and bcl-2 overexpressing cells (Bcl-2/6). **(C)** ELISA of PGE2 levels in CM derived from M14 control or bcl-2 overexpressing cells. **(D)** qRT-PCR and **(E)** ELISA analyses of IL-1β, IL-8 and IL-17 expression in M14 control and bcl-2 overexpressing cells. **(F)** qRT-PCR analysis of CCL2, CSF-1 and SDF-1 mRNA levels in M14 control and bcl-2 overexpressing cells. **(G)** Analysis of mRNA levels of IL-1β (IL-1R1, IL-1Ra and IL-1R2), IL-17 (IL-17RA), IL-8 (CXCR1) and CCL2 (CCR2) receptors in M-DM stimulated with CM from M14 control or bcl-2 overexpressing cells. The results are reported as % mRNA variation in macrophages exposed to CM derived from bcl-2/6 versus control one. (**B-G**) *p<0.05

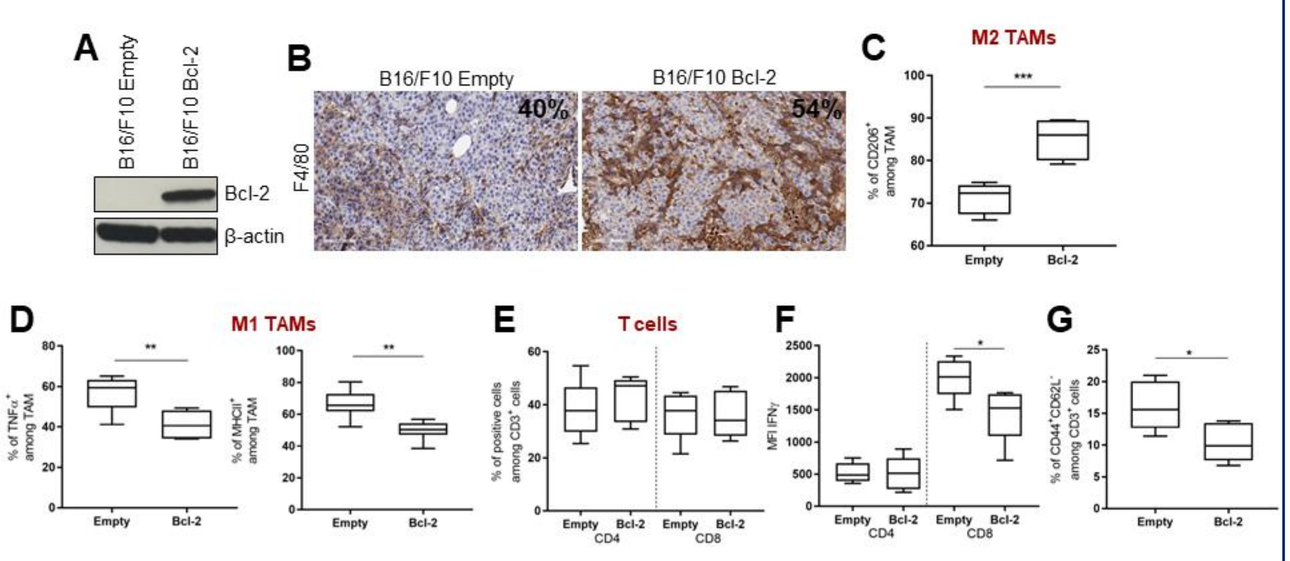
Bcl-2 overexpressing melanoma cells show increased expression and promoter activity of IL-1β, IL-17, RORa, RORc and CCL2 in a NF-kB-dependent manner



(**A**) ChIP analysis of p65 recruitment, **(B)** histone H3 acetylation at IL-1β, IL-8, COX-2 and CCL2 promoters in M14 control and two derivative bcl-2 overexpressing clones (Bcl-2/6, Bcl-2/37). (**C**) qRT-PCR analysis of RORa and RORc mRNA levels in M14 control and Bcl-2/6 cells. (**D**) Western Blot analysis of IKBα expression in M14 control cells, their derivative Bcl-2 overexpressing clone (Bcl-2/6) and Bcl-2/6 overexpressing IKBSR cells (Bcl-2/6 IKBSR). (**E**) qRT-PCR analysis of IL-1β, CCL2, IL-17, RORa, RORc, IL-8 and COX-2 expression in M14 control, Bcl-2/6 and Bcl-2/6 IKBSR cells.

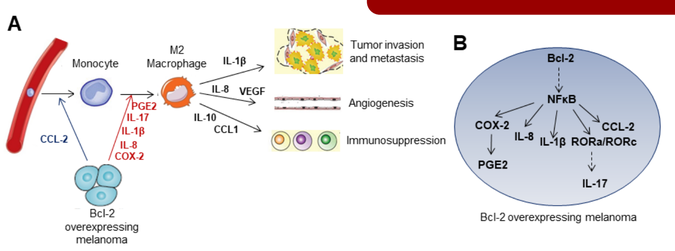
(**F**) qRT-PCR analysis of CD206, CCL1, CCL22, IL-10, COX-2 and IL-12 expression in human M-DM after exposure to CM from M14 control, Bcl-2/6 or Bcl-2/6 IKBSR cells. (**A-F**) *p<0.05.

Bcl-2 overexpressing melanoma tumors positively affect macrophage recruitment to the tumour site



(**A**) Western Blot analysis of bcl-2 expression in murine melanoma B16/F10 control (Empty) or bcl-2 overexpressing cells. (**B**) Representative images of IHC detection of F4/80 in B16/F10 empty and bcl-2 overexpressing tumors performed 15 days after cell injection in mice. Quantification by cytofluorimetric analysis of (**C**) $CD206^+$, (**D**) $TNF\alpha^+$ and $MHCII^+$ cells among tumor-associated macrophage (TAM) in B16/F10 control or bcl-2 overexpressing tumors. Quantification by cytofluorimetric analysis of (**E**) $CD4^+$ and $CD8^+$ (**F**) IFNγ production and (**G**) $CD44^+CD62L^-$ among $CD3^+$ infiltrating cells. (**C-G**) *p<0.05; **p<0.01; ***p<0.001.

Conclusions



Our findings show that tumor-specific bcl-2 controls a citokines-driven axis of macrophage diversion that could favours melanoma progression.