Semaphorin 5A drives melanoma progression: role of Bcl-2, miR-204 and c-Myb

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Background

Melanoma, the most aggressive form of skin cancer, is characterized by high rates of metastasis, drug resistance and mortality. Semaphorins (SEMAs), are involved in different physiological and developmental functions, including regulation of the nervous and immune systems, and angiogenesis. Controversial data exist regarding the role of Semaphorin 5A (Sema5A) in cancer, and particularly its role in melanoma has not been investigated.

Aim

In this work we investigated the role of Sema5A on the properties associated with melanoma progression and the factors involved in Sema5A regulation. We also focused on the functional relation between Sema5A and Bcl-2, an antiapoptotic protein associated with melanoma progression, resistance to apoptosis and poor prognosis.

Results

Sema5A is expressed in melanoma cell lines and metastatic specimens, and affects *in vitro* melanoma cell invasion, migration and vasculogenic mimicry

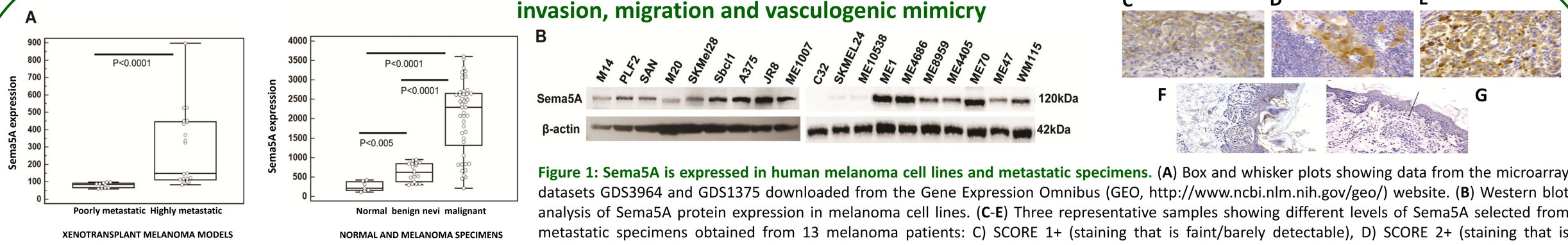

Figure 1: Sema5A is expressed in human melanoma cell lines and metastatic specimens. (**A**) Box and whisker plots showing data from the microarray datasets GDS3964 and GDS1375 downloaded from the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) website. (**B**) Western blot analysis of Sema5A protein expression in melanoma cell lines. (**C**-**E**) Three representative samples showing different levels of Sema5A selected from metastatic specimens obtained from 13 melanoma patients: C) SCORE 1+ (staining that is faint/barely detectable), D) SCORE 2+ (staining that is weak/moderate), E) SCORE 3+ (staining that is intense/strong. (**F,G**) Representative images from "in situ" melanoma specimen showing no immunoreactivity (F) or focal positivity (G) for Sema5A expression. Arrow indicates positive cell.

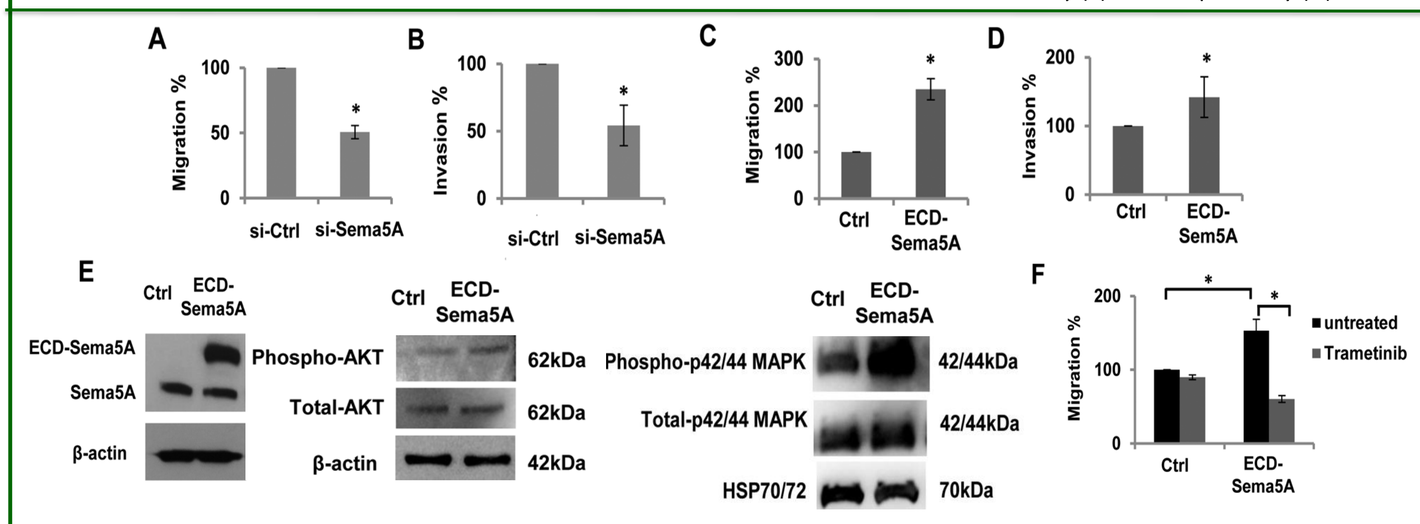

Figure 2: Sema5A affects *in vitro* melanoma cell migration and invasion. *In vitro* (**A**) migration and (**B**) invasion of M14 cells transfected with si-Ctrl or si-Sema5A. *In vitro* (**C**) migration and (**D**) invasion of M14 cells transfected with empty vector (Ctrl) or vector expressing the Sema5A protein (ECD-Sema5A). (**E**) Western blot analysis of Sema5A, total and phosphorylated AKT, total and phosphorylated p44/42 protein expression in M14 cells transfected with Ctrl or ECD-Sema5A vectors. (**F**) *In vitro* cell migration of M14 cells transfected with Ctrl or ECD-Sema5A, untreated or treated with 10nM Trametinib. **A-D, F** **p-values* <0.05

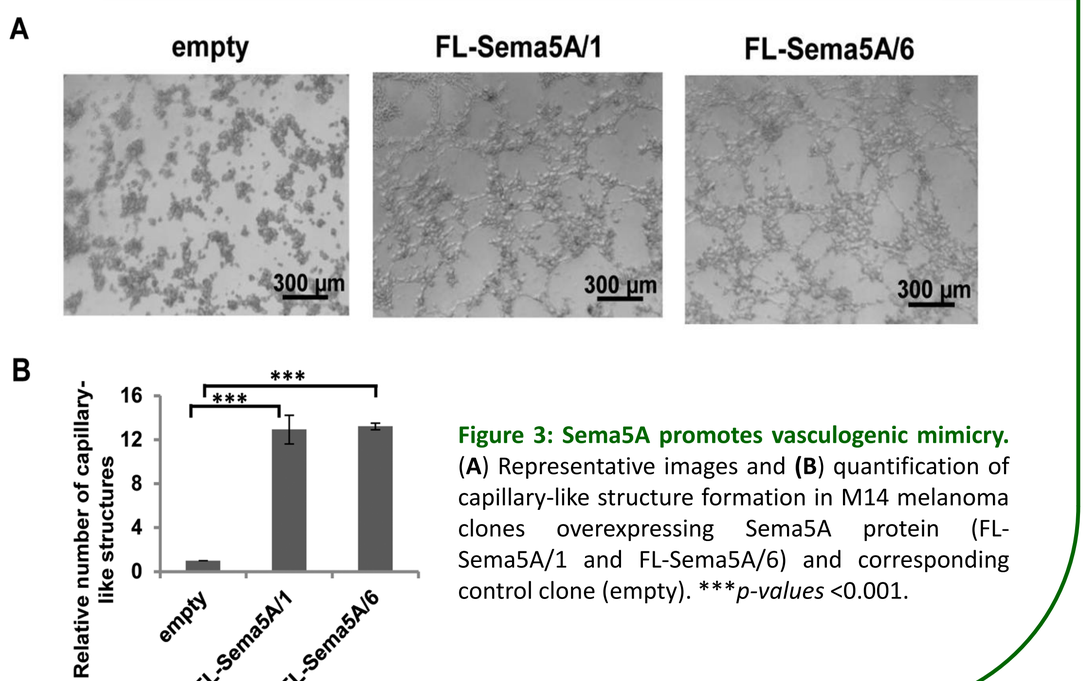

Figure 3: Sema5A promotes vasculogenic mimicry. (**A**) Representative images and (**B**) quantification of capillary-like structure formation in M14 melanoma clones overexpressing Sema5A protein (FL-Sema5A/1 and FL-Sema5A/6) and corresponding control clone (empty). ****p-values* <0.001.

Sema5A is regulated by the miR-204/c-Myb axis

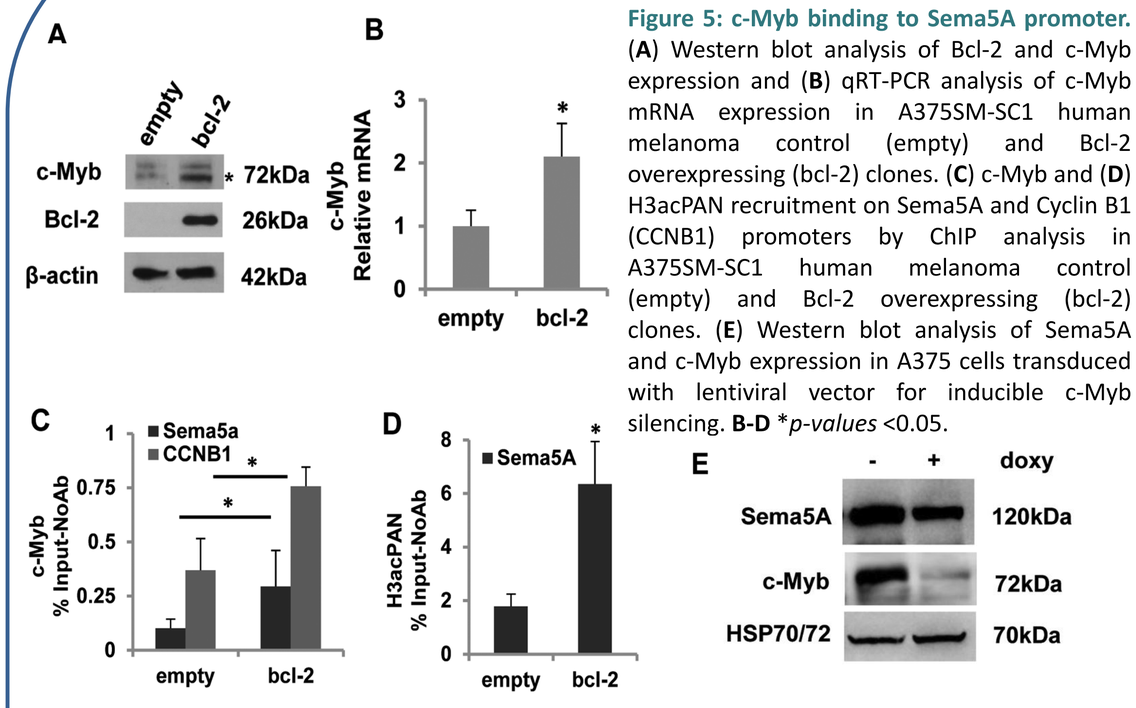

Figure 5: c-Myb binding to Sema5A promoter. (**A**) Western blot analysis of Bcl-2 and c-Myb expression and (**B**) qRT-PCR analysis of c-Myb mRNA expression in A375SM-SC1 human melanoma control (empty) and Bcl-2 overexpressing (bcl-2) clones. (**C**) c-Myb and (**D**) H3acPAN recruitment on Sema5A and Cyclin B1 (CCNB1) promoters by ChIP analysis in A375SM-SC1 human melanoma control (empty) and Bcl-2 overexpressing (bcl-2) clones. (**E**) Western blot analysis of Sema5A and c-Myb expression in A375 cells transduced with lentiviral vector for inducible c-Myb silencing. **B-D** **p-values* <0.05.

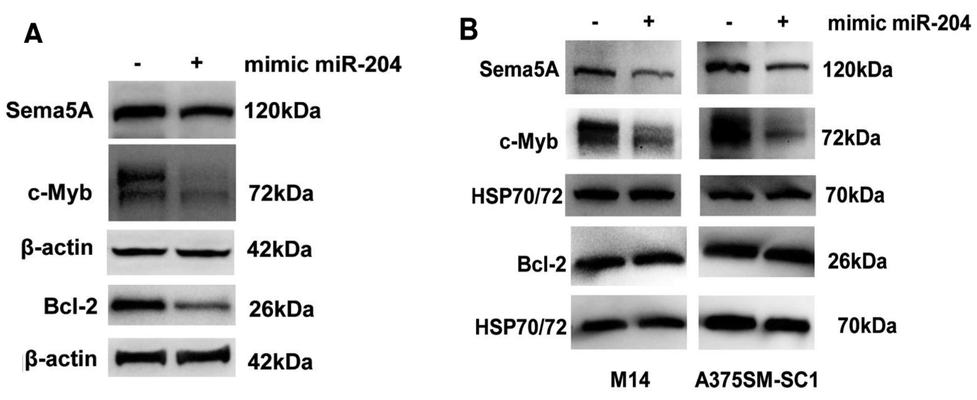

Figure 6: Sema5A is regulated by the miR-204/c-Myb axis. (**A**) Western blot analysis of Sema5A, Bcl-2 and c-Myb expression after mir-204 transfection in M14 parental cells. (**B**) Western blot analysis of Sema5A, Bcl-2 and c-Myb expression after miR-204 transfection in Bcl-2 overexpressing (bcl-2) clones from M14 and A375SM-SC1 cells.

Bcl-2 modulates Sema5A expression in melanoma models

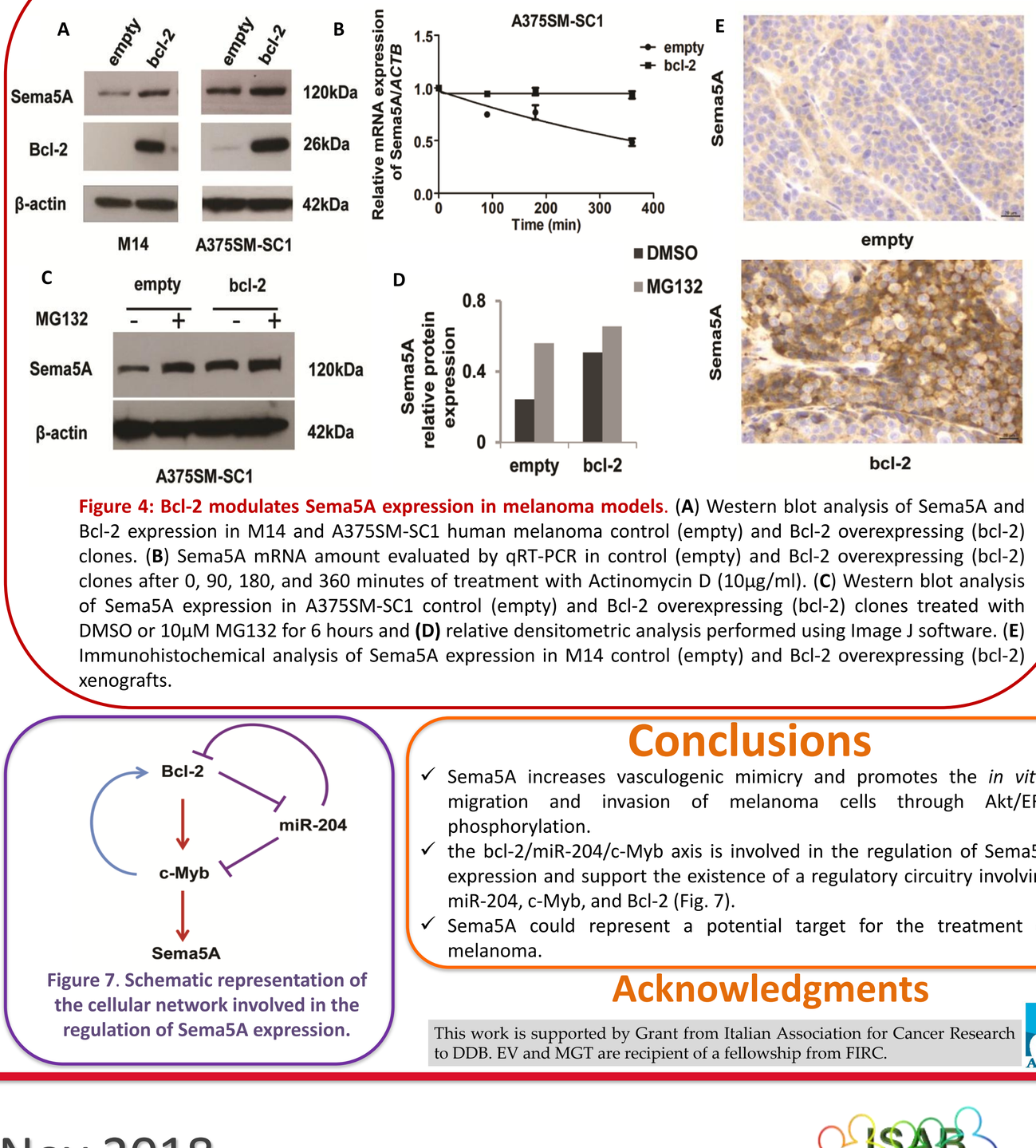

Figure 4: Bcl-2 modulates Sema5A expression in melanoma models. (**A**) Western blot analysis of Sema5A and Bcl-2 expression in M14 and A375SM-SC1 human melanoma control (empty) and Bcl-2 overexpressing (bcl-2) clones. (**B**) Sema5A mRNA amount evaluated by qRT-PCR in control (empty) and Bcl-2 overexpressing (bcl-2) clones after 0, 90, 180, and 360 minutes of treatment with Actinomycin D (10μg/ml). (**C**) Western blot analysis of Sema5A expression in A375SM-SC1 control (empty) and Bcl-2 overexpressing (bcl-2) clones treated with DMSO or 10μM MG132 for 6 hours and **(D)** relative densitometric analysis performed using Image J software. (**E**) Immunohistochemical analysis of Sema5A expression in M14 control (empty) and Bcl-2 overexpressing (bcl-2) xenografts.

Figure 7. Schematic representation of the cellular network involved in the regulation of Sema5A expression.

Conclusions

- ✓ Sema5A increases vasculogenic mimicry and promotes the *in vitro* migration and invasion of melanoma cells through Akt/ERK phosphorylation.
- ✓ the bcl-2/miR-204/c-Myb axis is involved in the regulation of Sema5A expression and support the existence of a regulatory circuitry involving miR-204, c-Myb, and Bcl-2 (Fig. 7).
- ✓ Sema5A could represent a potential target for the treatment of melanoma.

Acknowledgments

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