INVESTIGATING THE PREDICTIVE AND PROGNOSTIC ROLE OF SEMAPHORIN 5A IN MELANOMA PATIENTS AND ITS ROLE IN RESPONSE TO THERAPY

Matteo Brignone[1], Piera Rizzolo[2], Fabio Valenti[3], Marta Di Martile[1], Elisabetta Valentini[1], Martina Chiacchiarini[1], Marica Di Caprio[1], Patrizio Giacomini[4], Carlo Cota[2], Francesca Paolini[5], Chiara Mandoj[6], Tommaso Mancuso[6], Michelangelo Russillo[7], Andrea Sacconi[4], Isabella Manni[8], Donatella Del Bufalo[1], Simona D'Aguanno[1]

[1] Preclinical Models and New Therapeutic Agents Unit, IRCCS Regina Elena National Cancer Institute, Rome; [2] Genetic Research, Dermatological Molecular Biology and Dermatopathology Unit, IRCCS San Gallicano Dermatological Institute, Rome, Italy; [3] Ricerca Traslazionale Oncologica, IRCCS Regina Elena National Cancer Institute, Rome, Italy; [4] Clinical Trial Center, Biostatistics and Bioinformatics, IRCCS Regina Elena National Cancer Institute, Rome, Italy ; [5] Tumor Immunology and Immunotherapy Unit, IRCCS Regina Elena National Cancer Institute, Rome, Italy; [6] Clinical Pathology and Cancer Biobank, IRCCS Regina Elena National Cancer Institute, Rome, Italy; [7] First Division of Medical Oncology, IRCCS - Regina Elena National Cancer Institute, Rome, Italy; [8] SAFU Unit, IRCCS Regina Elena National Cancer Institute, Rome, Italy

E-mail: simona.daguanno@ifo.it; matteo.brignone@ifo.it

P304

Background

1. Melanoma

Melanoma represents the most malignant type of skin cancer, with increasing incidence worldwide. The introduction of BRAF and MEK inhibitors and immunotherapy has drastically improved the prognosis of melanoma patients, even though therapeutic resistance eventually ensues, resulting in disease progression and death. Of note, imitations in therapeutic options are still present for patients without BRAF mutations or in relapse from current treatments. Thus, the urgent need to identify predictive and prognostic molecules, and to find novel druggable targets for additional strategy of melanoma treatment.

2. Semaphorins

Semaphorins (SEMAs) are a family of 20 secreted or trans-membrane proteins with cleavable extracellular domain (**Fig.1**), initially identified for their pivotal role in neuronal plasticity. More recently, most of them have been reported to be involved in various aspects of tumor development and progression, and response to therapy (Valentini et al J Exp Clin Cancer Res. 2021 Apr 15;40(1):131).

3. Semaphorin 5A

In vitro, it has been demonstrated that Semaphorin 5A (Sema5A) promotes cell migration and invasion properties of melanoma cells through Akt/ERK activation, favours the formation of capillary-like structures, and its expression is regulated by Bcl-2 and the miR-204/c-Myb axis (D'Aguanno et al. J Exp Clin Cancer Res. 2018 Nov 19;37(1):278.).

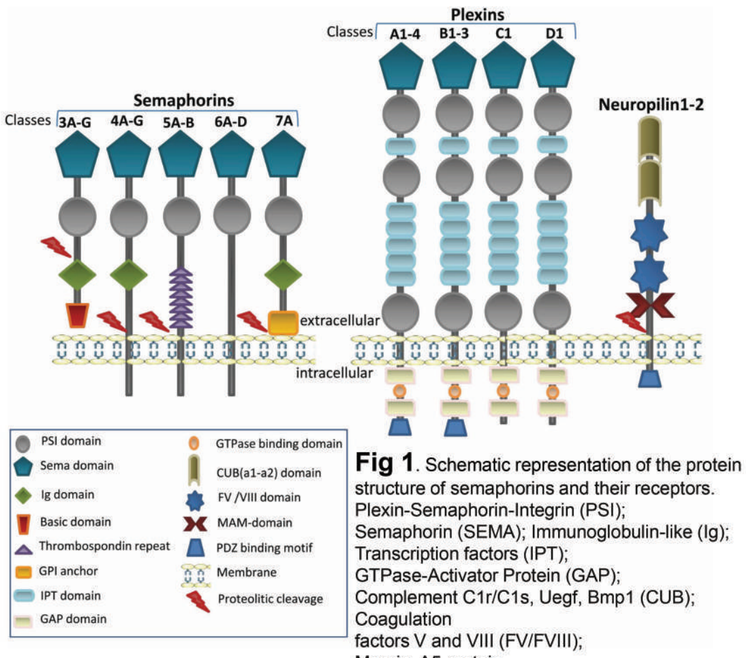


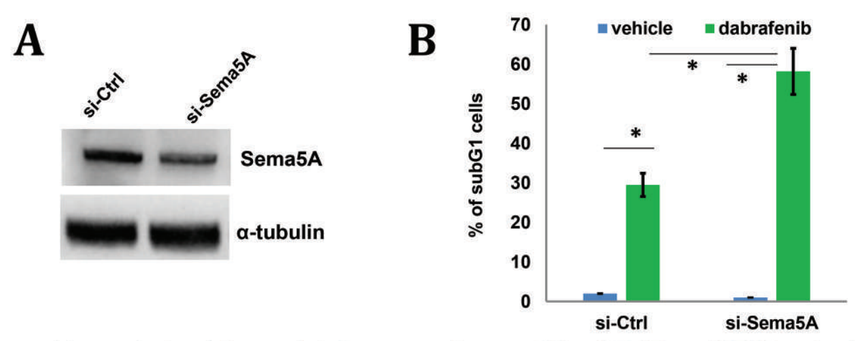
Fig 1. Schematic representation of the protein structure of semaphorins and their receptors. Plexin-Semaphorin-Integrin (PSI); Semaphorin (SEMA); Immunoglobulin-like (Ig); Transcription factors (IPT); GTPase-Activator Protein (GAP); Complement C1r/C1s, Uegf, Bmp1 (CUB); Coagulation factors V and VIII (FV/FVIII); Meprin, A5 protein, receptor protein tyrosine phosphatase Mu (MAM); PSD95/Dlg/ZO-1 (PDZ)

Aims

On the basis of these consideration, by using preclinical and melanoma patient biological samples (biopsies and sera), the aims of the project are: i) to investigate the role of Sema5A in the response to therapy; ii) to investigate the predictive and prognostic role of Sema5A (and other SEMAs).

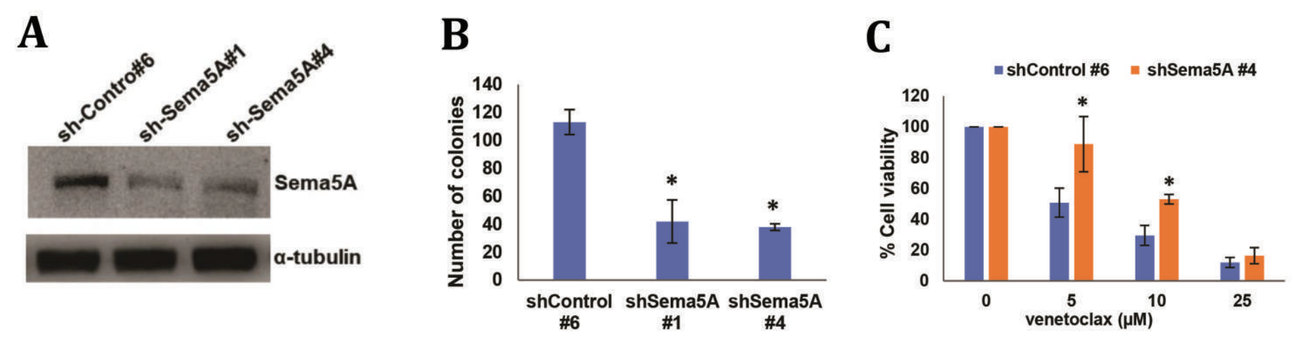
Results

1. Sema5A downregulation sensitizes to dabrafenib



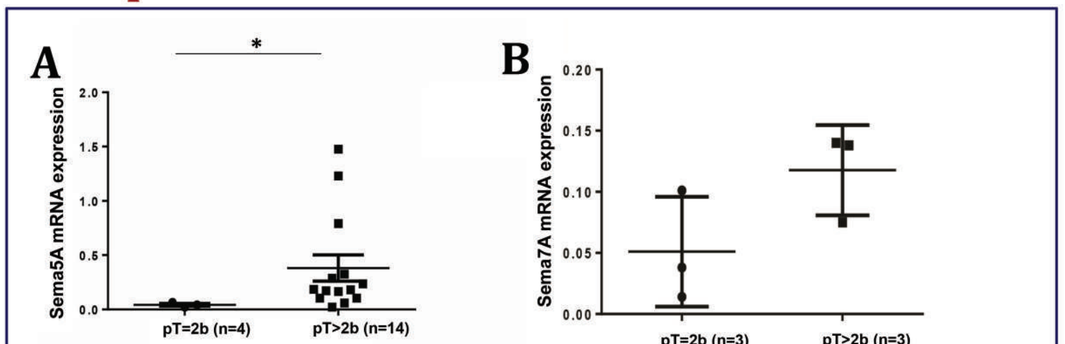
A) Western blot analysis of Sema5A in human melanoma M14 (BRAF mut/NRAS wt) cells transfected with siRNA directed against Sema5A (si-Sema5A), or against scramble sequence (si-Ctrl). α tubulin was evaluated as control of equivalent transfer and loading. **B**) Flow cytometric quantification of cells in the subG1 peak of cell cycle distribution by propidium iodide staining in M14 cells transfected with si-Sema5A or si-Ctrl, treated with BRAF inihibitor dabrafenib (0,5 μM,48h)

2. Sema5A downregulation reduces clonogenic ability and increses resistance to venetoclax



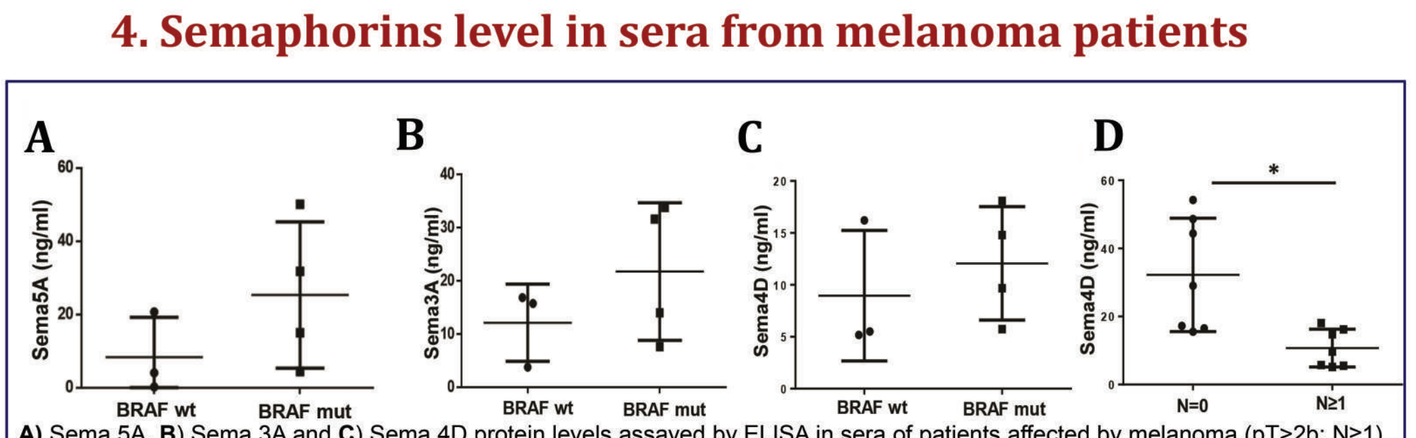
A) Western blot analysis of Sema5A in murine melanoma B16/F10 (BRAF wt/NRAS wt) cells transduced with lentiviral vector for Sema5A silencing (#1 and #4) respect to cells transduced with control vector (shControl), α tubulin was evaluated as control of equivalent transfer and loading. **B**) quantification of clonogenic ability evaluated in shControl#6, shSema5A#1 and shSema5A# 4 cells; *p< 0.05. **C**) Analysis of cell viability by MTT assay in shControl# 6 and shSema 5 A# 4 clones treated with venetoclax, a specific Bcl-2 inhibitor, at indicated concentration for 72h. (calculated IC50=11.86 μM for shSema5A#4; IC50=4.5 μM for shControl#6). The results are reported as "viability of treated cells/viability of control cells×100, *p< 0.05.

3. Sema5A and Sema7A are more expressed in specimens from advanced melanoma



A) Sema5A and **B**) Sema7A mRNA expression analysed by qRT-PCR in pT=2b and pT>2b melanoma specimens collected at IFO-ISG, within the Melanoma 4P project. *p< 0.05.

4. Semaphorins level in sera from melanoma patients



A) Sema 5A, **B**) Sema 3A and **C**) Sema 4D protein levels assayed by ELISA in sera of patients affected by melanoma (pT>2b; N≥1) segregated in BRAF wt (n=3) and BRAF mut (n=4)). **D**) Sema 4D protein levels in sera of patients with positive lymph node (N≥1; n=7) respect to patients with negative lymph node (N=0, n=7); *p<0,05. Biological samples have been collected at IFO-ISG, within the Melanoma 4P project.

Conclusions

Preliminary results indicate that: i) *in vitro,* Sema5A protein level could affect responce to drug treatments; ii) higher level of Sema5A transcript has been detected in patients with advanced melanoma, also a similar trend has been observed for Sema7A; iii) in sera samples of patients collected at T0 (time of lymph node evaluation), classified on BRAF status, an increased trend of Sema5A, Sema3A, and 4D level was observed in patients with mutated BRAF gene, respect to patients carrying the wild type gene. Finally, we observed a significant reduction of Sema4D protein in sera of patients with positive lymph node at diagnosis respect to patients with negative lymph node. Further investigations are necessary to confirm results obtained *in vitro* and in biological samples.