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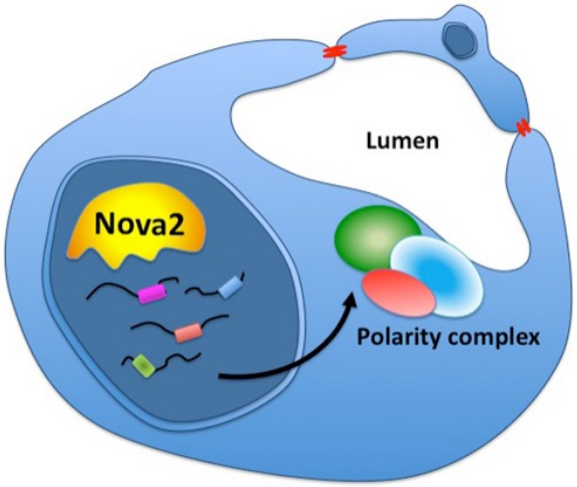
Research activity. Alternative splicing (AS) is the process producing different mature transcripts (mRNAs) from a single primary RNA (pre-mRNA). AS decisions are modulated by a number of *cis*-acting sequence motifs on the pre-mRNA bound by *trans*-acting splicing regulatory factors (SRFs) that function in a coordinate manner to promote or inhibit the inclusion of specific exons.
Nearly all of human protein-coding genes undergoes AS giving rise to different protein isoforms with distinct structural and functional properties, diverse localization or stability. Interestingly, the majority of human AS events displays cell type-, tissue type- or condition-specific regulation. Hence, AS represents one of the major mechanisms to expand the coding potential of our genome in order to establish and maintain fundamental properties in development and tissue identity.
AS has a direct role in promoting cancer progression. Altered expression of specific SRFs allow neoplastic cells to generate cancer-specific AS isoforms involved in tumor establishment, progression and resistance to therapeutic treatments. For this reason, SRFs and cancer-specific AS events are a promising source of new diagnostic, prognostic or therapeutic tools for human cancer.

Post-transcriptional regulation of angiogenesis



Angiogenesis (the formation of new blood vessels form from pre-existing vessels) is a hallmark of solid tumors allowing oxygen and nutrients to reach proliferating cancer cells and providing tumor cells with the metastatic route to colonize distant organs. While the number of anti-angiogenic agents developed for cancer treatment has risen over the past decade, anti-angiogenic monotherapies led only to mild clinical benefits.
The mechanism and the relevance of AS for the regulation of angiogenesis are still largely unknown. The identification of AS isoforms and SRF with important roles in the tumor vasculature will be crucial to identify novel and more efficient anti-angiogenic therapies for cancer treatment.

In the past, we discovered that NOVA2, a tissue-restricted AS factor previously characterized for its important functions in the neuronal cells, is also expressed in the vascular endothelium. From genome-wide approaches and histological analysis of NOVA2 mutant animals generated through CRISPR/Cas9 technology, we demonstrated that NOVA2 plays a relevant role in angiogenesis during embryonic vascular development. In addition, we identified novel genes regulated at the levels of AS by NOVA2 in ECs and we characterized the vascular role of a number of novel NOVA2 splicing targets. Our recently published results also showed that NOVA2 is overexpressed in tumor vasculature, with negligent expression in others cell types within the tumor. Notably, NOVA2 has a prognostic value in tumor progression, as its levels correlate with shorter overall survival of cancer patients. To the best of our knowledge, NOVA2 is the only SRF specifically upregulated in cancer vasculature.

Our main interests are:
Project 1. Understand the relevance of NOVA2 for tumor angiogenesis and to identify novel pathways relevant for cancer progression. We generated mice with NOVA2 knockout only in ECs (**in collaboration with Serena Zacchigna, ICGEB of Trieste**), in which tumor growth and angiogenesis will be study.

Project 2. Characterize the molecular mechanism(s) sustaining NOVA2 upregulation in tumor blood vessels. We have identified candidate transcription factors controlling NOVA2 expression levels in the tumor vasculature.

Project 3. To study the interplay between NOVA2 and other AS regulators in ECs. We have collected important data supporting a hierarchy of splicing factors integrating AS decisions during angiogenesis.

Project 4. Identify novel AS isoforms generated by NOVA2-mediated AS and specifically expressed in tumor vasculature to develop innovative therapeutic strategies (**in collaboration with Benjamin Blencowe, University of Toronto; Raffaella Giavazzi, Ist. Mario Negri of Milan**). We have purified ECs from tumor cancer patients, which have been depleted of NOVA2 to perform RNA-seq experiments.

Selected publications
1) Pradella D, Deflorian G, Pezzotta A, Di Matteo A, Belloni E, Campolungo D , Paradisi A, Bugatti M, Vermi W, Campioni M, Chiapparino A, Scietti L, Forneris F, Giampietro C, Volf N, Rehman M, Zacchigna S, Paronetto MP, Pistocchi A, Eichmann A, Mehlen P, **Ghigna C**. A ligand-insensitive UNC5B splicing isoform regulates angiogenesis by promoting apoptosis.
Nat Commun. 2021;12: 4872. **IF= 14.919**

2) Di Matteo A, Belloni E, Pradella D, Cappelletto A, Volf N, Zacchigna S, **Ghigna C**. Alternative splicing in endothelial cells: novel therapeutic opportunities in cancer angiogenesis. *J Exp Clin Cancer Res.* 2020; 39:275. **IF= 11.161**

3) Belloni E, Di Matteo A, Pradella D, Vacca M, Wyatt CDR, Alfieri R, Maffia A, Sabbioneda S, **Ghigna C**. Gene Expression Profiles Controlled by the Alternative Splicing Factor Nova2 in Endothelial Cells. *Cells* 2019; 8:1498. **IF= 6.6**

4) Angiolini F, Belloni E, Giordano M, Campioni M, Forneris F, Paronetto MP, Lupia M, Brandas C, Pradella D, Di Matteo A, Giampietro C, Jodice G, Luise C, Bertalot G, Freddi S, Malinverno M, Irimia M, Moulton JD, Summerton J, Chiapparino A, Ghilardi C, Giavazzi R, Nyqvist D, Gabellini D, Dejana E, Cavallaro U, **Ghigna C**. A novel L1CAM isoform with angiogenic activity generated by NOVA2-mediated alternative splicing. *eLife* 2019; 8:e44305. **IF= 8.140**

5) Pradella D, Naro C, Sette C, **Ghigna C**. EMT and stemness: flexible processes tuned by alternative splicing in development and cancer progression. *Molecular Cancer* 2017; 16:8. **IF= 27.401**

6) Giampietro C, Deflorian G, Gallo S, Di Matteo A, Pradella D, Bonomi S, Belloni E, Nyqvist D, Quaranta V, Confalonieri S, Bertalot G, Orsenigo F, Pisati F, Ferrero E, Biamonti G, Fredrickx E, Taveggia C, Wyatt CD, Irimia M, Di Fiore PP, Blencowe BJ, Dejana E, **Ghigna C**. The alternative splicing factor Nova2 regulates vascular development and lumen formation. *Nat Commun*. 2015; 6:8479. **IF= 14.919**