MELANOMA GROUP

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OVERVIEW

Melanomas are inherently aggressive cancers for which basic and translational research have significantly improved patient prognosis. Nevertheless, clinical responses are still incomplete. The long-term goals of our Group are to identify new progression biomarkers and therapeutic agents. Focusing on stress response programmes involving apoptosis, autophagy and endosome mobilisation, we have discovered lineage-specific oncogenes that define the melanoma 'fingerprint'. Transcriptomic and proteomic analyses of the melanoma secretome have allowed us to define how tumour cells remodel the (lymp)angiogenic vasculature and avoid immune recognition. Moreover, we have generated a unique set of animal models for non-invasive imaging of melanoma progression *in vivo*. These systems have led to the validation of nanoparticle-based treatments which are being currently being tested in clinical trials. Our ultimate objective is to improve the management of patients with otherwise refractory metastatic melanomas.

"We have developed the first-in-class lymphoreporter mouse models of melanoma for *in vivo* imaging and pharmacological testing of new metastatic agents."

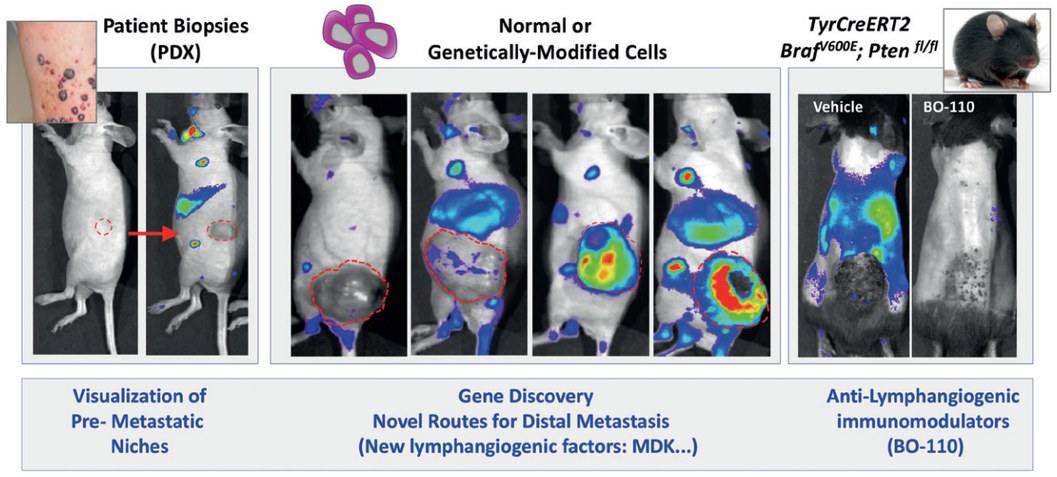

Figure 1 *Vegfr3*^{LUC} (MetAlert) mice to test PDX, melanoma cell lines or genetically engineered mice for the visualisation of pre-metastatic niches and as a platform for gene discovery and drug testing (here shown to visualise the efficacy of the dsRNA-based nanoparticle BO-110).

CNIO Melanoma Group: Objectives and model systems

Melanomas are aggressive solid tumours and a prime example of how integrated basic and clinical research has significantly improved patient prognosis. Nevertheless, despite great success with targeted and immune-based therapies, sustained clinical responses are still limited. Moreover, the field lacks molecular markers of diagnosis, and the knowledge on how melanomas progress and metastasise is largely incomplete. In addition, one of the main hurdles to advance in this disease is the lack of animal models to monitor melanoma initiation and progression *in vivo*.

To this end, our Group focuses on three main areas of research (FIGURE 1):

- → **Aim 1.** Oncogenic pathways selectively deregulated in melanoma that may represent new diagnostic indicators.
- → **Aim 2.** Risk factors and prognostic markers.
- → **Aim 3.** Animal models that allow for non-invasive monitoring of pre-metastatic niches.

Lineage-specific oncogenic dependencies in melanoma

One of the long-term objectives of the Melanoma Group is to discover **new melanoma drivers**. We have previously identified a cluster of endolysosomal-associated genes that distinguish melanoma from over 35 additional malignancies (Alonso-Curbelo *et al.*, *Cancer Cell* 2014; Alonso-Curbelo *et al., Oncotarget* 2015a and *Oncotarget* 2015b). Further analyses of lysosomal-dependent pathways also revealed unique features of autophagy genes (ATG5) in melanoma (García-Fernández *et al., Autophagy* 2016). Additional contributions of autophagy to melanoma cell survival and response to targeted therapy were generated in collaboration with the Ashani Weeraratna group at the Wistar Institute (USA) (Ndoye *et al., Cancer Res* 2017).

Other melanoma-enriched regulatory mechanisms were identified by focusing on RNA binding proteins (RBPs). We selected RBPs (a family of over 950 members) because they are largely unexplored in melanoma, although this is a tumour characteristically associated with a plethora of changes in mRNA gene expression profiles. Performing a series of genome wide studies (i.e. genomic, transcriptomic, proteomic and interactomic analyses) we uncovered new roles of the RBPs CPEB4 and CUGBP1 in the modulation of mRNA stability, with unexpected targets involving master specifiers of the melanocyte lineage (Perez-Guijarro *et al.*, *Nat Commun* 2016; Cifdaloz *et al.*, *Nat Commun* 2017).

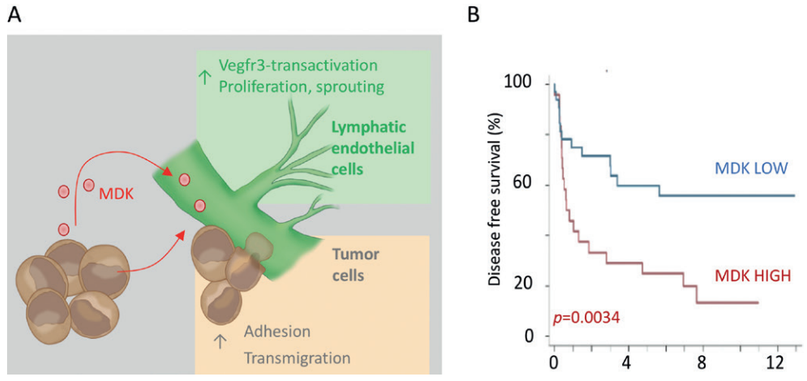

Figure 2 MIDKINE (MDK) as pro-metastatic driver and marker of poor prognosis in melanoma. (**A**) Schematic depicting newly-described roles of tumour-secreted MDK on tumour cells and the lymphatic vasculature. (**B**) Kaplan-Meier survival curves of melanoma patients (Stage II-IV) classified on the basis of MDK expression in sentinel lymph nodes.

'MetAlert' mice for the visualisation of premetastatic niches in melanoma and as a platform for gene discovery and target validation

We have also made great progress regarding one of the most pressing needs in the melanoma field, namely, the mechanisms that enable melanoma cells to disseminate already from lesions of barely 1 mm in depth. In collaboration with Sagrario Ortega at the CNIO, we have generated a series of mouse models of melanoma that have the unique feature of revealing how these cells act 'a distance' from very early stages of tumour development, activating the lymphatic vasculature and preparing metastatic niches before their colonisation (Olmeda *et al.*, *Nature* 2017; see the versatility of these mice for gene discovery and pharmacological analyses in FIGURE 1). Using these 'MetAlert' animals we found the growth factor MIDKINE as a new driver of lymphangiogenesis and melanoma metastasis (FIGURE 2, A). The physiological relevance of these data was validated in human clinical biopsies where MIDKINE expression correlated with poor patient prognosis (FIRGURE 2, B). This paper of Olmeda *et al.* was highlighted in *Nature*, *Cancer Discovery*, *Developmental Cell* and other scientific journals, and was awarded the *Premio "Constantes y Vitales"* by the *Fundación AtresMedia* for the Best Publication in Biomedical Research in 2017. This article was also considered as being among the *Top 10 publications in Spain in 2017* by the news agency *EFE*. This publication, together with others from the Soengas Group, was recognised by the *Estela Medrano Memorial Award* by the *Society of Melanoma Research*, which honours the most influential female leaders in the melanoma field. In addition, clinical trials with the compound BO-112 performed by the biotechnology company *Bioncotech Therapeutics* were considered as being among the *14 Most Relevant Scientific Hits in 2017* by the *SINC* agency (the Spanish *Information and Scientific News Service*). BO-112 is a derivative of the polyplex BO-110 generated at the CNIO by the Soengas laboratory. ■

PUBLICATIONS

- Olmeda D, Cerezo-Wallis D, Riveiro-Falkenbach E, Pennacchi PC, Contreras-Alcalde M, Ibarz N, Cifdaloz M, Catena X, Calvo TG, Cañón E, Alonso-Curbelo D, Suarez J, Osterloh L, Graña O, Mulero F, Megías D, Cañamero M, Martínez-Torrecuadrada JL, Mondal C, Di Martino J, Lora D, Martinez-Corral I, Bravo-Cordero JJ, Muñoz J, Puig S, Ortiz-Romero P, Rodriguez-Peralto JL, Ortega S, Soengas MS (2017). Whole body imaging of lymphovascular niches identifies premetastatic roles of midkine. *Nature* 546, 676-680.
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  Cancer Discovery Research Watch (2017). A lymphoreporter mouse model identifies midkine as a metastasis driver. *Cancer Discovery*, doi: 10.1158/2159-8290.CD-RW2017-126.
  Karaman S, Alitalo K (2017). Midkine and melanoma metastasis: a malevolent mix. *Dev Cell* 42, 205-207.
  Pérez-Guijarro E, Merlino G (2017). Lymphangiogenesis: from passive disseminator to dynamic metastatic enabler. *Pigment Cell Melanoma Res,* doi: 10.1111/pcmr.12621.
- Soengas MS, Patton EE (2017). Location, location, location: spatio-temporal cues that define the cell of origin in melanoma. *Cell Stem Cell* 21, 559-561.
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- Cifdaloz M, Osterloh L, Graña O, Riveiro-Falkenbach E, Ximénez-Embún P, Muñoz J, Tejedo C, Calvo TG, Karras P, Olmeda D, Miñana B, Gómez-López G, Cañon E, Eyras E, Guo H, Kappes F, Ortiz-Romero PL, Rodríguez-Peralto JL, Megías D, Valcárcel J, Soengas MS. (2017). Systems analysis identifies melanoma-enriched pro-oncogenic networks controlled by the RNA binding protein CELF1. *Nat Commun* 8, 2249.
- Ndoye A, Budina-Kolomets A, Kugel CH 3rd, Webster MR, Kaur A, Behera R, Rebecca VW, Li L, Brafford PA, Liu Q, Gopal YNV, Davies MA, Mills GB, Xu X, Wu H, Herlyn M, Nicastri MC, Winkler JD, Soengas MS, Amaravadi RK, Murphy ME, Weeraratna AT (2017). ATG5 mediates a positive feedback loop between Wnt signaling and autophagy in melanoma. *Cancer Res* 77, 5873-5885.
- Riveiro-Falkenbach E, Ruano Y, García-Martín RM, Lora D, Cifdaloz M, Acquadro F, Ballestín C, Ortiz-Romero PL, Soengas MS, Rodríguez-Peralto JL (2017). DEK oncogene is overexpressed during melanoma progression. *Pigment Cell Melanoma Res* 30, 194-202.

AWARDS AND RECOGNITION

- Estela Medrano Memorial Award for the most influential female melanoma researcher, the Society for Melanoma Research, USA.
- "Constantes y Vitales" Award for the Best Publication in Medicine, *Fundación Atresmedia,* Spain.
- "Premio Executivas de Galicia 2017", *Executivas de Galicia*, Spain.
- Finalist "*Premios MAS Mujeres a Seguir*", Spain.
- Olmeda *et al., Nature* 2017; considered one of the Top 10 scientific publications in Spain in 2017, *Agencia EFE*.