CELL DIVISION AND CANCER GROUP

Marcos Malumbres
Group Leader

Staff Scientists
Mónica Álvarez, Guillermo de Cárcer (until September)

Post-Doctoral Fellows
Dario Hermida, Begoña Hurtado,

Carolina Maestre, María Salazar, Carolina Vilarroya

Graduate Students
Ana F. Batalha Martins, José González, María Maroto (until September), Diego Martínez, Beatriz Salvador, María Sanz (until January)

Technicians
Aicha El Bakkali (since September), Verónica García (since March) (TS)*, Beatriz Ortigosa (PEJ, CAM) **, Elisabet Zapatero (TS) *

Titulado Superior (Advanced Degree)
***Plan de Empleo Joven de la Comunidad de Madrid* (Youth Employment Plan, Community of Madrid)

Visiting Scientists
Senn Wakahashi (since December) (Kobe University, Japan), Yucheng Zhang (Jilin University, China)

OVERVIEW

The Cell Division and Cancer Group is interested in deciphering the mechanisms by which cell division and cell proliferation are regulated in mammalian cells. During the last years, we have used different mouse models to understand the relevance of cell cycle regulators, including cell cycle kinases and phosphatases, as well as proteins involved in ubiquitin-dependent degradation, in the control of cell division and tissue physiology. Our interests are: i) to understand the basic control mechanisms that regulate the cell division cycle; ii) to characterise the physiological and therapeutic consequences of cell cycle deregulation; iii) understanding self-renewal and pluripotency in stem cell biology and tumour development; and iv) to find and validate new targets for cancer therapy. As a final goal, we aim to generate information that may be useful to improve therapeutic strategies against cancer cell proliferation.

"During 2018, we investigated the relevance of PLK1 and MASTL as oncogenes and as therapeutic targets in breast cancer, as well as the effects of MASTL mutations in patients with thrombocytopenia."

RESEARCH HIGHLIGHTS

The Mastl-PP2A axis in the cell cycle and cancer

Cell cycle progression is typically triggered by phosphorylation of a large number of proteins involved in different cellular pathways. Several families of protein kinases involved in cell cycle progression, such as Cyclin-dependent kinases (CDKs) or Polo-like kinases (PLK), have been thoroughly studied over the last few decades. However, the identity and relevance of phosphatases is less well-established. Recently, the cell cycle kinase Mastl (also known as Greatwall) emerged as a key player in cell cycle control by inhibiting the PP2A phosphatase during mitosis. Mastl phosphorylates 2 small proteins, endosulfine (ENSA) and ARPP19, which in their phosphorylated form bind and inhibit PP2A-B55 complexes, thus contributing to the phosphorylation of mitotic phosphoproteins (FIGURE 1).

However, its physiological relevance in normal tissue homeostasis or disease is less known. After an initial screening in several tumour types, we found that MASTL was upregulated in a significant fraction of breast tumours and correlated with poor prognosis in breast cancer patients (a collaboration with M.A. Quintela, CNIO; and C. Caldas, Cancer Research UK). Importantly, genetic downregulation or ablation of MASTL, either using RNA interference or CRISPR-Cas systems, resulted in defective proliferation of a subset of breast cancer cells, both *in vitro* and *in vivo*, suggesting the therapeutic potential of inhibiting this kinase in breast cancer (a collaboration with the Oncology R&D group at Pfizer; Álvarez-Fernández *et al.*, *Cell Death Differ* 2018).

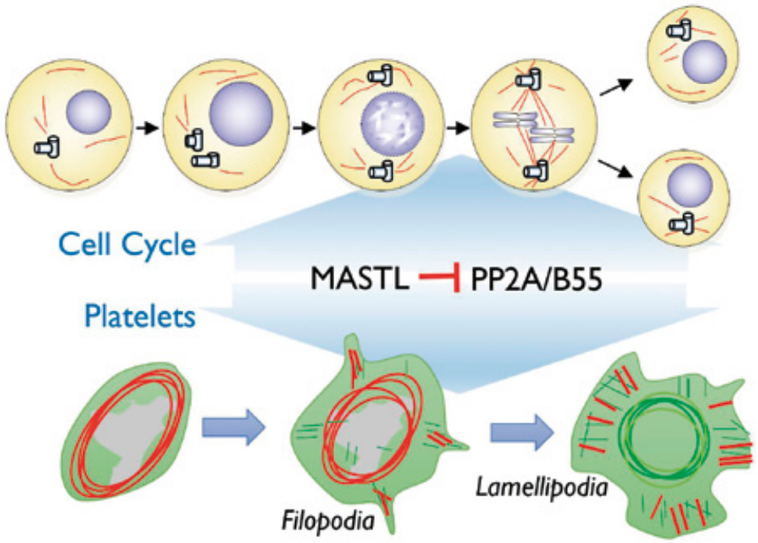

Figure 1 A dual role for the MASTL-PP2A/B55 pathway in cell cycle progression and actin cytoskeleton dynamics. During the cell cycle, MASTL inhibits PP2A/B55 to prevent dephosphorylation of mitotic phosphoresidues. In postmitotic cells such as platelets, the same pathway is involved in the control of cytoskeleton dynamics by modulating phosphoresidues in the signalling pathways that control the reorganisation of the actin cytoskeleton.

Before the function of MASTL in mitosis was proposed, the corresponding human gene was found mutated in patients with thrombocytopenia. To understand the effects of this mutation we recently generated a knockin mouse model carrying that mutation (Mastl E166D in the mouse). Mastl E166D mice developed thrombocytopenia but, unexpectedly, this defect was not due to abnormal cell cycle in megakaryocytes but to defective activation of mutant platelets. In the presence of this mutation, PP2A is constitutively inhibited resulting in hyperphosphorylation of proteins involved in actin cytoskeleton signalling during platelet activation. Mastl E166D mutant platelets were prematurely activated and displayed defective morphology and function, as well as decreased survival, thus contributing to thrombocytopenia (Hurtado *et al.*, *J Clin Invest*, in press). These data uncovered a new function of MASTL in the actin cytoskeleton in postmitotic cells, entailing important implications in human disease.

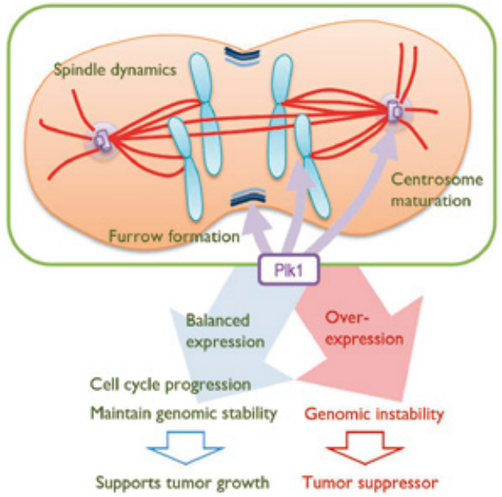

Figure 2 A central role for Plk1 in controlling genomic stability. Plk1 controls several processes during the cell cycle, including centrosome maturation, spindle dynamics and the formation of the cytokinesis furrow. In normal conditions these functions support cell proliferation and tumour growth, and inhibiting Plk1 may prevent tumour cell proliferation. However, when Plk1 is overexpressed, tumour cells are genomically unstable resulting in defective growth of breast cancers.

Plk1: oncogene or tumour suppressor

Among the multiple kinases involved in cell cycle progression, PLK1 is considered an attractive cancer target and a few small-molecule inhibitors are currently under evaluation in clinical trials. However, our knowledge about the relevance of this protein in adult mammalian tissues is still limited. In 2017, we described a critical role for the mouse Plk1 in controlling the contraction of smooth muscle cells and blood pressure (de Cárcer *et al.*, *Nat Med* 2017), suggesting possible toxic effects linked to the inhibiting of this kinase that need to be controlled in patients. More recently, we evaluated to what extent the expression levels of Plk1 contribute to tumour development in mouse models. Although Plk1 is frequently considered as an oncogene, we observed that Plk1 overexpression prevented proper cell proliferation by generating genomic aberrations in polyploid and aneuploid cells (FIGURE 2). Overexpression of Plk1 impaired breast cancer development induced by Kras or HER2 oncogenes, thereby suggesting a tumour suppressor function for this protein in these models (a collaboration with R. Sotillo, German Cancer Research Center; de Cárcer *et al.*, *Nat Commun* 2018). Specifically, in human breast cancer, PLK1 overexpression correlated with better prognosis. Although these data do not argue against the use of PLK1 inhibitors in the clinic, they add new levels of knowledge that will be critical when optimising the use of mitotic inhibitors in cancer therapy. ■

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