HISTOPATHOLOGY CORE UNIT

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**Plan de Empleo Joven* (Youth Employment Plan)

OVERVIEW

Pathology is the branch of science devoted to the study of the structural, biochemical and functional changes in cells, tissues and organs underlying disease. The Histopathology Unit offers support and expertise through a full range of services covering paraffin embedding and tissue sections to histochemical stains; research and diagnostic immunohistochemistry (IHC) testing; antibody validation; *in situ* hybridization techniques (including *in situ* detection of mRNAs by RNAScope); and the generation of tissue microarrays. Furthermore, the Unit offers other value-added services implemented by a team of highly specialised technicians, such as laser-capture microdissection; slide digitalisation; image analysis; and quantification. The Unit collaborates with CNIO researchers in the histopathological characterisation of animal models of disease, providing them with the required pathology expertise. In addition, the Unit offers its portfolio of services to other institutions, including hospitals, research centres and private companies.

"In 2021, despite the Covid-19 pandemic, the Unit was able to return to its usual levels of workload and services, even exceeding expectations in some specific areas."

RESEARCH HIGHLIGHTS

In 2021, the Unit was able to return to the standard levels of workload and services recorded before the pandemic and, in some specific areas, such as immunohistochemistry and image digitalisation and analysis, even exceeded expectations. Thus, more than 26,000 paraffin blocks of tissue samples were generated, and *ca.* 21,000 techniques were performed, including histological and IHC techniques (with dual and triple staining being increasingly in demand), *in-situ* chromogenic hybridization, tissue microarrays, slide scanning, etc.

During 2021, we made significant progress in digitalising our material, with approximately 35 % of all the slides generated converted to digital files. In addition, 10% of these were subjected to image analysis and quantification.

We also consolidated the *in situ* hybridization technology for mRNA detection (RNAScope), with 160 cases analysed, some of them with double staining, using the Ventana-Roche automatic platform for IHC stains. This new technique enables efficient detection of specific mRNAs directly on sections from formalin-fixed paraffin-embedded (FFPE) tissues, thus providing a spatial dimension to gene expression analysis. The applications of this new technology are manifold, e.g., as an alternative to IHC whenever it is difficult to find specific antibodies working well on FFPE tissues, or to validate results from other technologies, among others.

The high quality of the techniques run by the Unit continues being endorsed by External Quality Assessment Schemes. In this respect, our histochemical techniques were evaluated by UK NEQAS. Similarly, NordiQC and SEAP have evaluated a subset of our IHC techniques under different modules, including general markers, breast cancer markers and PD-L1; these all obtained good scores.

Training and outreach activities are also a critical component of the Unit's activities. Although some of the usual activities in this area were compromised due to the pandemic, the Unit was still able to participate in a Master's course on oncology research, in online format, and we hosted a pre-doctoral student for a short training stay on immunohistochemistry techniques during the last quarter of the year. ■

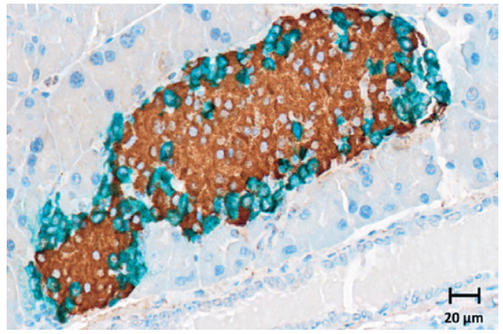

FIGURE Example of dual IHC staining. The image shows a picture of an islet of Langerhans in the pancreas, with double staining for insulin (DAB, brown) and glucagon (Teal, blue). It can be seen that the insulin staining is homogeneous across the islet, whereas glucagon is localised more in the periphery of the islet.

PUBLICATIONS

- Sanclemente M *et al.* (incl. Caleiras E, Barbacid M) (2021). RAF1 kinase activity is dispensable for KRAS/p53 mutant lung tumor progression. *Cancer Cell* 39, 294-296.
- De la Calle Arregui C *et al.* (incl. de Martino A, Caleiras E, Campos-Olivas R, Mulero F, Muñoz J, Efeyan A) (2021). Limited survival and impaired hepatic fasting metabolism in mice with constitutive Rag GTPase signaling. *Nat Commun* 12, 3660.
- Salmón M *et al.* (incl. Caleiras E, Muñoz J, Ortega S, Barbacid M) (2021). KRAS4A induces metastatic lung adenocarcinomas in vivo in the absence of the KRAS4B isoform. *Proc Natl Acad Sci USA* 118, e2023112118.
- Ortega-Molina A *et al.* (incl. Caleiras E, Efeyan A) (2021). Inhibition of Rag GTPase signaling in mice suppresses B cell responses and lymphomagenesis with minimal detrimental trade-offs. *Cell Rep* 36,109372.
- Santos M *et al.* (incl. Caleiras E, Robledo M, Rodriguez-Antona C) (2021). Prevalence of pathogenic germline variants in patients with metastatic renal cell carcinoma. *Genet Med* 23, 698-704.
- Compte M *et al.* (incl. Martínez-Torrecuadrada J, González-García P) (2021). Case report: an EGFR-targeted 4-1BB-agonistic trimerbody does not induce hepatotoxicity in transgenic mice with liver expression of human EGFR. *Front Immunol* 11, 614363.
- Mouron S *et al.* (incl. Caleiras E, Quintela-Fandino M, Bueno MJ) (2021). FGFR1 amplification or overexpression and hormonal resistance in luminal breast cancer: rationale for a triple blockade of ER, CDK4/6, and FGFR1. *Breast Cancer Res* 23, 21.
- Luna-Dulcey L *et al.* (incl. Caleiras E, Quintela-Fandino M) (2021). [6]-Gingerol-derived semi-synthetic compound SSi6 inhibits tumor growth and metastatic dissemination in triple-negative breast cancer xenograft models. *Cancers (Basel)* 13, 2855.
- Monteagudo M *et al.* (incl. Megías D, Caleiras E, Roncador G, Blasco MA, Robledo M) (2021). Analysis of telomere maintenance related genes reveals NOP10 as a new metastatic-risk marker in pheochromocytoma/paraganglioma. *Cancers (Basel)* 13, 4758.