HUMAN CANCER GENETICS PROGRAMME

JAVIER BENÍTEZ Programme Director

The Human Cancer Genetics Programme (HCGP) is a translational research programme working on areas related to genetics, genomics, pharmacogenetics, molecular cytogenetics and the environmental bases of human cancer. The HCGP works in close collaboration with the clinical community.

Currently, the HCGP is composed of three Research Groups and three Units. The Human Genetics Group, led by Javier Benítez, focuses on contributing to the understanding of the genetic bases of some hereditary tumours. Mercedes Robledo leads the Hereditary Endocrine Cancer Group that aims to identify new major susceptibility genes related to hereditary endocrine tumours as well as to define markers associated with differences in anticancer drug response and toxicity. Both Groups are also involved in the search for low susceptibility alleles that explain sporadic cancers. The Genetic and Molecular Epidemiology Group, led by Núria Malats, works not only from a genetic but also from a non-genetic point of view. She analyses exogenous factors that contribute to explain, together with genetic factors (low susceptibility alleles), the susceptibility to pancreatic and bladder cancer. The Genotyping Unit, headed by Anna González-Neira, supports our three research groups from a technical point of view, and provides support to other CNIO groups as well as to external users. They also work in pharmacogenetics within the framework of their own line of research. The Molecular Cytogenetics and Genome Editing Unit, headed by Sandra Rodríguez-Perales, contributes to this provision of support with classical and molecular cytogenetics techniques and with new genome editing technologies. In addition, her research is focused on the design of human stem cell models carrying cytogenetic alterations. Finally, the Familial Cancer Unit coordinates the clinical part of the Programme through the CNIO Familial Cancer Consultancy, which is located at the *Hospital de Fuenlabrada*. Miguel Urioste is responsible for these activities and leads a research line focused on hereditary colorectal cancer.

The Programme collaborates closely with the clinical community, not only to foster cooperation in genetic diagnosis but also to promote training and education. This year the Familial Cancer Consultancy attended around 550 consultancies, performed 1,417 genetic diagnoses and carried out 1,290 cytogenetic studies. In addition, the Programme's Groups have hosted 6 resident physicians from different Spanish hospitals who rotated in the Groups and Units for 3-month periods. We also offer professionals from different national and international research centres the opportunity to join us, either as visitors or for training visits consisting of short-term stays of 1-3 months (a total of 6 international and 10 national visitors were hosted in 2018). In terms of education, 1 foreign and 10 national Master's students and 9 national PhD students have worked on their research projects, 1 of whom has already successfully defended their thesis.

“ We use different omics and epidemiologic studies to achieve our goals; this is combined with functional studies that validate our results. Finally, we translate our conclusions into clinical practice.”

Finally, one of the main objectives of the Programme is to establish research collaborations with national and international groups; this is well demonstrated by our publication record as well as the key roles held by several of the Programme's members in consortia and international projects. Currently, we collaborate with 14 international Consortia that are representative of the main types of tumours that we focus on. In addition, we participate in 2 international projects from Europe.

Summary of milestones and major achievements during 2018:

- → Mercedes Robledo: the identification of DLST as a new peochromocytoma and/or Paraganglioma (PPGL) susceptibility gene.
- → Anna González-Neira: the identification of pharmacogenetic variants predicting response to neoadjuvant single-agent doxorubicin or docetaxel.
- → Núria Malats: interaction of FHC and smoking increases pancreatic cancer risk.
- → Javier Benítez: the identification of three susceptibility genes *PLEC, EXO5 and DNAH7* as novel susceptibility genes in testicular cancer.
- → Sandra Rodríguez-Perales: gene editing cancer therapy project, selected by CaixaImpulse Programme in the 2018 edition.
- → Mercedes Robledo: became member of the ENS@T Steering Committee (European Network for the Study of Adrenal Tumours).
- → Javier Benítez's Group: was accepted in the international Consortium of Testicular Cancer.

HUMAN GENETICS GROUP

Javier Benítez
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Post-Doctoral Fellow
Oriol Calvete

Graduate Students
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Technicians
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Titulado Superior (Advanced Degree)

OVERVIEW

We have continued to decipher the genetic bases of hereditary and sporadic breast cancer. In addition, we participated in a project that combines the genotype and the phenotype in order to stratify and select women at high risk of developing breast cancer. Other families with rare tumours are also the object of our studies, for example, testicular cancer whose genetic bases are unknown. More recently, we started working on a study to elucidate the common genetic origin of different autoimmune-originated pathologies: gastric neuroendocrine tumours or chronic atrophic gastritis plus different immune diseases in other tissues, such as thyroiditis, diabetes or arthritis. We have identified several genes thereby opening up new avenues for new treatments. Finally, we have progressed in understanding the role of glycosylase genes as modifiers of hereditary breast cancer and their role along the cell cycle.

"We have discovered 3 new genes that confer susceptibility to testicular cancer and a moderate breast cancer susceptibility gene. A whole pathway with several genes associated to gastric neuroendocrine tumours or chronic atrophic gastritis plus several immune diseases, has been identified."

RESEARCH HIGHLIGHTS

Deciphering the role of rare variants in breast cancer

The European project BRIDGES, in which we participate, has 3 main phases. The first one was the study of 36 candidate genes in 60,000 breast cancer cases and controls in order to determine their role as possible moderate susceptibility genes. This part of the work has been completed and we have confirmed 20% of them. The second phase will involve the whole sequencing of these 60,000 cases, trying to discover new susceptibility genes. The work will start towards the end of year or the beginning of next year; our group will be responsible for coordinating this Work Package.

Breast cancer susceptibility genes

In a whole-exome sequencing study of 4 BRCAX families we identified a mutation in the moderate susceptibility gene *ATM* as being responsible for the disease in one of the families (Tavera-Tapia *et al.*, 2017). In a second family, we found a deleterious mutation in an excellent candidate gene *RECQL5* that belongs to a family of DNA helicases that have a role in the Homologous Recombination (HR) DNA repair pathway. Using a combination of targeted next-generation sequencing and genetic and functional assays, we identified 7 deleterious or likely deleterious mutations in the gene in a series of 700 BRCAX cases and only 1 deleterious mutation in 700 controls, suggesting that the gene could actually explain a small percentage of the BRCAX families (Tavera-Tapia *et al.*, submitted).

SNPs and the BER pathway

We investigated the molecular basis underlying the effect of an SNP in the DNA glycosylase *UNG* as an ovarian cancer risk modifier in *BRCA2* mutation carriers (Baquero *et al.*, submitted). We found that an SNP rs34259 is associated with significant UNG down-regulation and a better performance of the enzyme, measured by a lower accumulation of uracil at the telomeres in *BRCA2* mutation carriers. Our findings could help to explain the association of this variant with a lower cancer risk in *BRCA2* mutation carriers. In addition, we want to study the role of this and 2 other glycosylases previously studied, OGG1 and NEIL2, across the cell cycle.

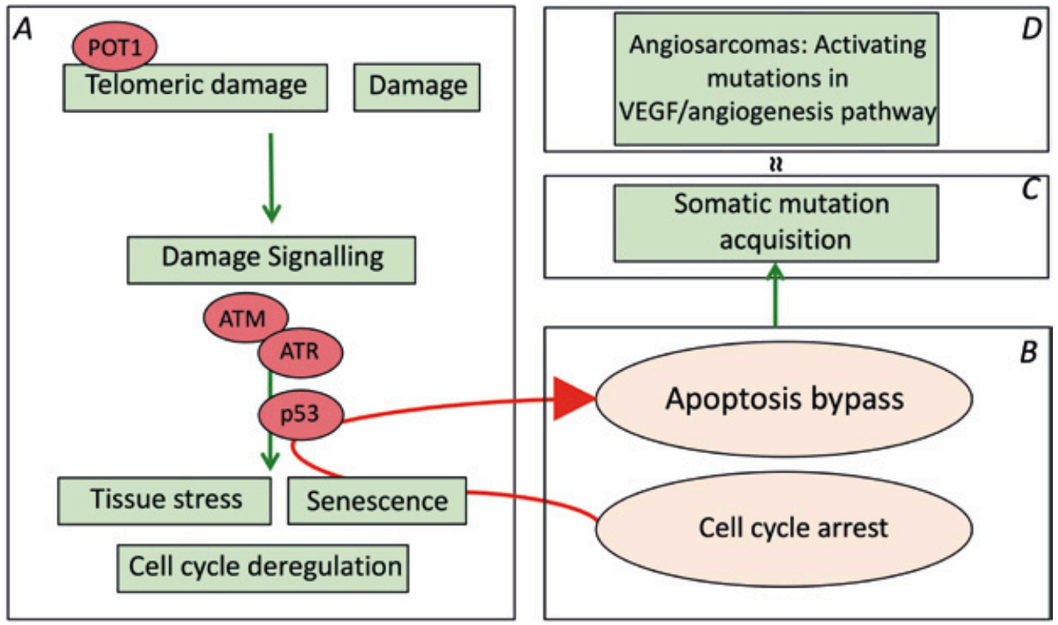

Figure Tumorigenic mechanisms for cardiac angiosarcomas (CAS). (**A**) Mutations found in *POT1* and other genes involved in the damage response signalling (*ATR/ATM* and *TP53*), correlate with the constitutional deregulation of the cell cycle, which triggers senescence and tissue stress due to the depletion of the progenitor cells. (**B**) Malfunction of the tissue stress would arise a bypass in the apoptotic regulation. (**C**) Apoptosis bypass would fuel the acquisition of multiple somatic events. (**D**) In all the studied CAS (familial – carrying POT1 mutation – and sporadic CAS), somatic activating mutations were found in the angiogenesis pathway, which drives tumour formation.

Familial cancer exome project

In 2015, we identified a gene responsible for families with cardiac tumours (*POT1*) (Calvete *et al.*, 2015). Recently, we described its relation not only to cardiac tumours but also to other different types of tumours (Calvete *et al.*, 2016). We investigated why the malfunction of this gene involves not only abnormal telomere length but is also able to generate different tumours in different tissues in a similar way as P53. We analysed the somatic changes in several cardiac tumours with and without *POT* mutations and have concluded that the inhibition of *POT1* gene function, and the damage-response malfunction, would activate ATR-dependent DNA damage signalling, which increases cell cycle arrest in asymptomatic tissues and might interfere the apoptosis mechanism; this would permit the further acquisition of somatic mutations in the VEGF/angiogenesis pathway (POT1 deregulation) and other angiosarcomas (mutations in damage-signalling), which drives tumour formation. The same observation was made in sporadic cardiac lesions (Calvete *et al.*, submitted).

In 2015, we published the identification of the *ATP4A* gene as being responsible for families with gastric neuroendocrine tumours (Calvete *et al.*, 2015). In 2017, we extended this study to a new family that presented the same lesion along with hypothyroidism and arthritis. The family presented 2 mutations in *ATP4A* and *PTHR1* in a digenic model (Calvete *et al.*, 2017). We further explored the apparent relation of gastric autoimmune disease (gastric neuroendocrine tumour or chronic atrophic gastritis) plus a second immune disease; we found several mutations in new genes involved in homeostasis function (solute carriers) altering *ATP4A* function. We have designed a panel of 15 genes from this pathway and we are currently performing a screening in a large number of patients carrying these combinations. Several gastroenterologists, pathologists and endocrinologists are collaborating in this project.

During the past few years, we have been collecting families with testicular cancer and also sporadic tumours. We conducted a first study in 17 families with over 71 members and by whole exome sequencing we identified several candidate genes; 3 of them (PLEC, EXO5 and DNAH7) were validated in a large case-control association study (Paumard *et al.*, 2018). We then continued the study with more cases and separated 2 main histologic groups, seminomas and non-seminomas. We differentiated several altered pathways and the spermatogenesis pathway was significantly altered. We studied this pathway in depth and discovered a biomarker that differentiated familial, bilateral and sporadic cases, as well as seminomas from non-seminomas. ■

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HEREDITARY ENDOCRINE CANCER GROUP

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Technicians
Javier Lanillos (TS)*(since June), Rocío León

'Titulado Superior (Advanced Degree)

OVERVIEW

Our Group is mainly interested in identifying genetic risk factors involved in endocrine tumour susceptibility. Through a comprehensive analysis of tumour genomic features we have been able to propose diagnostic and prognostic markers, to identify altered pathways that could be therapeutically targeted, and to identify new major susceptibility genes.

We are also interested in defining markers associated with differences in anticancer drug response and toxicity. We are applying targeted and whole-exome next-generation sequencing to a large series of clinically well-characterised patients. The aim is to identify new therapeutic approaches to personalise cancer treatment. These efforts will collectively improve the diagnosis, prognosis and treatment of patients.

"We identified a new susceptibility gene for paraganglioma, discovered predictive markers of mTORi response, and uncovered the Hsa-miR-139-5p/HNRNPF axis as a critical modulator of thyroid tumour virulence."

RESEARCH HIGHLIGHTS

Recurrent germline *DLST* mutations in patients with multiple pheochromocytomas and paragangliomas (PPGLs). Taking as a starting point the involvement of the TCA cycle in PPGL development, we aimed to identify novel disease-related genes involved in this key metabolic pathway that could explain additional patients lacking mutations in known susceptibility genes. To this end, targeted sequencing of thirty-seven TCA cycle-related genes was applied to DNA from 104 PPGL patients with no mutations in the major known predisposing genes. In order to decipher the role of the identified variants, omic-based analyses, TCA-related metabolite determination and $^{13}C_5$-glutamate labelling assays were performed. We identified *DLST* germline variants in ~7% of patients. A recurrent mutation, p.Gly374Glu, found in 43% of patients, triggered accumulation of 2-hydroxyglutarate, both in tumours and in a heterologous cell-based assay designed to functionally evaluate *DLST* variants. p.Gly374Glu-DLST-mutated tumours exhibited loss of heterozygosity as well as consistent methylation and expression profiles. We also found positive DLST immunostaining not only in *DLST*-mutated tumours, but also in other tumours in which the TCA cycle was disrupted. In summary, this study reveals *DLST* as a new PPGL susceptibility gene and further strengthens the relevance of the TCA cycle in PPGL development.

Mutations leading to extraordinary responses to mTOR inhibitors. The inhibitors of the mammalian target of rapamycin (mTOR) are key drugs for the treatment of several tumours. However, we lack markers able to identify patients with enhanced treatment sensitivity. To discover molecular determinants of drug response and to contribute to the definition of predictive biomarkers, we recruited renal cancer patients with extraordinary responses to these drugs and performed a comprehensive genomic, immunochemical and functional characterisation of the tumours. In two young adults with metastatic cancer, a renal epithelioid angiomyolipoma (EAML) and a chromophobe renal cell carcinoma, that upon rapalog treatment had a complete response at metastatic sites and durable responses, we could identify *TSC2* somatic mutations as causative of the extraordinary responses. These findings support a high efficacy of mTOR inhibitors in malignant EAML and in a subset of patients with chromophobe renal cancer, and propose sequencing of mTOR pathway genes to guide therapy with these drugs.

Deep sequencing of small RNAs reveals a prognosis marker functionally associated with alternative splicing modulation in thyroid cancer. It is urgent to identify biomarkers and functional networks associated with aggressive thyroid cancer in order to anticipate disease progression and facilitate patient-personalised management. The miRnome sequencing of thyroid tumour series enriched for advanced disease patients uncovered miRnome profiles correlated with tumour-specific histopathological and molecular features, such as stromal-cell infiltration and tumour-driver mutation. Differential analysis considering disease prognosis revealed a consistent hsa-miR139-5p down-expression in primary carcinomas from patients with recurrent/metastatic disease. Exogenous expression of hsa-miR-139-5p significantly reduced migration and proliferation abilities of anaplastic thyroid cancer cells. Proteomics analysis pointed to *RICTOR*, *SMAD2/3* and *HNRNPF* as hsa-miR-139-5p putative targets *in vitro*.

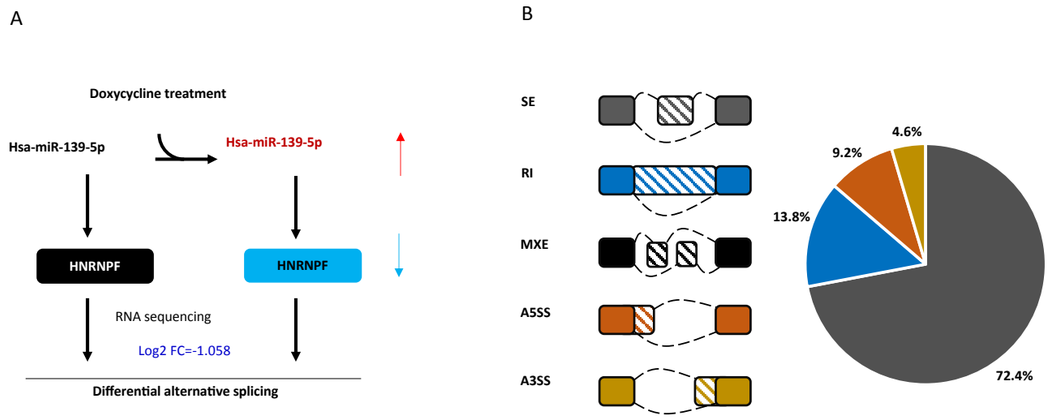

Figure Hsa-miR-139-5p/HNRNPF axis modulates gene-transcripts balance. (**A**) Alternative splicing analysis experiment. DeSeq2 differential expression analysis showed a reduction of *HNRNPF* mRNA abundance (Log2 FC=-1.058) upon hsa-miR139-5p expression induction. rMATS method identified differences in alternative splicing. (**B**) Events with a significant different inclusion level (FDR<0.05) upon hsa-miR-139-5p/HNRNPF axis regulation. Analysis considers junction and target exon counts from RNA sequencing data. SE: Skipped exon, MXE: Mutually exclusive, A5SS: Alternative 5' splice site, A3SS: Alternative 3' splice site; RI: Retained intron.

Significantly, mRNA abundance of *HNRNPF*, an alternative splicing factor mainly involved in cryptic exon inclusion/exclusion, showed an anti-correlation with hsa-miR-139-5p expression in human tumours. Analysis of alternative splicing from RNA sequencing data revealed 174 events differentially regulated upon *HNRNPF* repression in genes and signalling cascades critical for thyroid cancer (FIGURE). These results point at hsa-miR139-5p/HNRNPF/gene-transcripts balance as a novel regulatory axis associated with tumour virulence and modulation of major thyroid cancer signalling pathways. ■

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AWARDS AND RECOGNITION

- Mercedes Robledo, Group Leader of the 706 Unit, CIBERER (*Centro de Investigación Biomédica en Enfermedades Raras*). Research Programme on Hereditary Cancer, Haematological and Dermatological diseases.

GENETIC AND MOLECULAR EPIDEMIOLOGY GROUP

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Technicians
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Students in Practice
Pablo Alarcón (May-Oct., *Universidad de Vic*), Alba M. Fernández del Pozo (*Hospital 12 de Octubre*), Lisa Van Hoogstraten (until Aug., Radboud University Medical Center)

Visiting Scientists
Chiaka L. Anumudu (until Jan., University of Ibadan), Jesús López (Common Management Solutions), Isabel A. Martín (*Universidad CEU San Pablo*), Silvia Pineda (since June, UCSF)

OVERVIEW

The scope of the research carried out by our Group ranges from the identification of aetiological agents and mechanisms, to the translation of the findings into the clinical and Public Health domains, focusing on bladder, pancreatic, and breast cancers.

We employ a wide variety of biomarkers to better characterise exposures, genetic susceptibility patterns, and cancer outcomes. *Omics* data provide a unique opportunity in this regard and the Group explores its integration in epidemiologic studies.

The strategic goals of the Group are to:

→ Identify non-genetic and genetic factors, as well as their interactions, associated with cancer development and progression and with its molecular/omics subphenotypes.
→ Develop and apply statistical/informatics tools to model the risk, prediction, and clinical course of patients with cancer by integrating epidemiologic with *omics* information.
→ Assess clinical and public health strategies for cancer control using current genomic tests and data.

"The integration of omics and non-omics data in the same risk models poses several challenges and demands of appropriate analytical strategies. We are contributing to this field towards a personalised prevention of cancer."

RESEARCH HIGHLIGHTS

Research findings

In 2018, the Group mainly focussed its research on pancreatic cancer while building resources for bladder cancer research. For **pancreatic cancer** (PC), we continued exploiting the data generated by the PanGenEU Study to further characterise pancreatic cancer risk. Two main articles exemplify our contributions to this domain. First, by applying complementary analytical approaches we reported that, regardless of non-genetic risk factors, the risk of PC was 2.5 higher among family members with more than 2 relatives affected with PC, with this risk being stronger in current smokers (FIGURE 1). Furthermore, we confirmed that PC was diagnosed at younger ages among those subjects with a family history of PC who smoked than in non-smokers. In the second article, we reported on the underlying genetic basis behind PC and its associated multimorbidities network through a computational approach using the DisGeNET. This strategy allowed us to identify several autoimmune diseases linked to PC and the shared altered genes (FIGURE 2). These associations were subsequently confirmed at the individual level in the PanGenEU study population of 1,705 PC cases and 1,084 controls that resulted in a reduced risk of PC in subjects having ≥2 autoimmune diseases. These findings again pointed to the role of the immunological status in PC carcinogenesis. We also continued to participate in international large-scale investigations to further characterise the genetic susceptibility and somatic alteration landscape of PC. For **bladder cancer** (BC), the Group reported on the inverse association between asthma and BC using the Spanish Bladder Cancer/EPICURO Study resources. This reduced risk of BC was especially observed among aggressive tumours. The Group also participated in the discovery and validation of both urine and tumour prognostic marker combination in large European studies of non-muscle invasive BC. We also

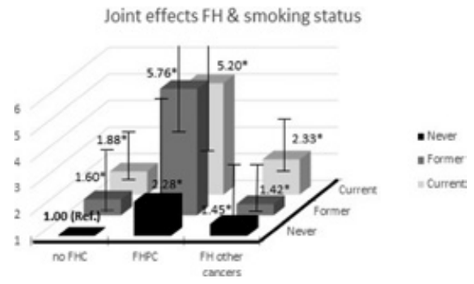

Figure 1 Odd ratios for the joint effect of family history of cancer (FHC) / Family history of pancreatic cancer (FHPC) and smoking on pancreatic cancer risk. PanGenEU case-control study.

performed a review of the genetic susceptibility to BC risk and progression based on GWAS hits. Most of the variants were common and conferred small risk and, therefore, they were not clinically actionable at the individual level.

Methodological contributions

The Group made contributions to both integrative analytic approaches considering omics and non-omics (OnO) data as well as in the nutrition epidemiological filed. Regarding the latter, we compared the antioxidant profiles of 21 a priori-defined Mediterranean diet indexes and reported that the level of dietary antioxidant intake captured through the different indexes differed due to the variation in their construction. As of the data integrative efforts, we observed that only a small number of published studies performed a 'real' integration of OnO data, primarily to predict cancer outcomes. We identified the challenges in OnO data integration and presented, discussed, and proposed integrative analytical strategies towards its integration.

Translational activities

The Group actively provides support in several clinical trials on immunotherapy and vitamin D in bladder cancer at the methodological level. We continue to sustain the Spanish Familial PC Registry (PanGen-FAM) and the establishment of the European Registry of PC (PancreOS). We lead the Research Work Stream of the Pancreatic Cancer Europe (EPC) multistakeholder platform, with who we hosted a session on PC Liquid Biopsy during the 2018 ESMO GI Meeting. To increase awareness of PC among health policy makers and discuss the urgent need to invest in PC research, we participated and co-organised sessions with MEPs at the European Parliament and with delegates at the Annual Meeting of the European Alliance of Personalized Medicine. ■

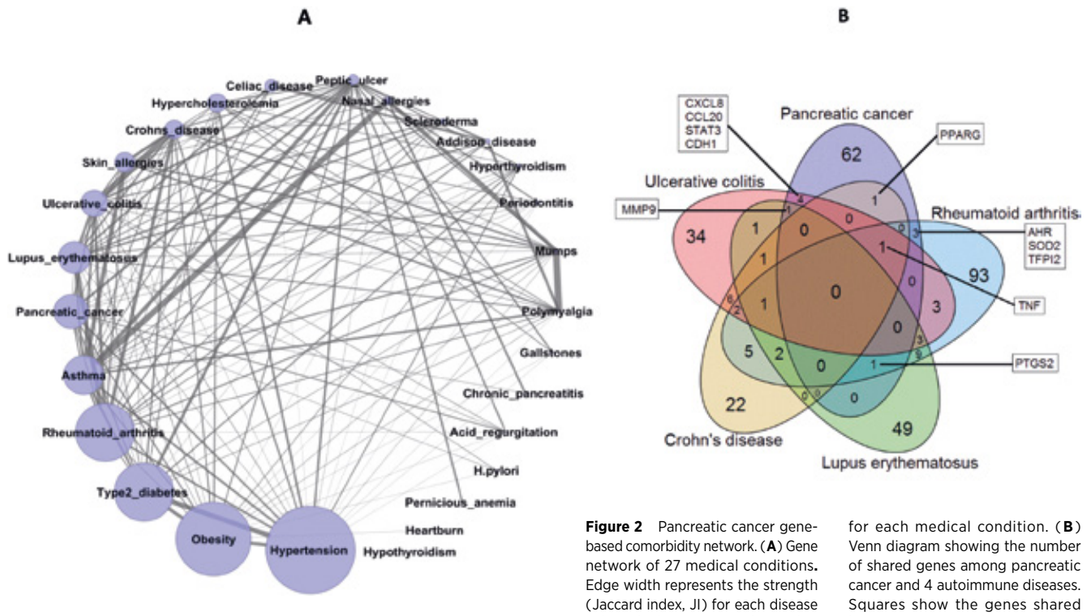

Figure 2 Pancreatic cancer gene-based comorbidity network. (**A**) Gene network of 27 medical conditions. Edge width represents the strength (Jaccard index, JI) for each disease pair. Node size represents the number of genes obtained through DisGeNET for each medical condition. (**B**) Venn diagram showing the number of shared genes among pancreatic cancer and 4 autoimmune diseases. Squares show the genes shared between pancreatic cancer and autoimmune conditions.

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AWARDS AND RECOGNITION

- Member of the jury of the Banco Sabadell Award.

FAMILIAL CANCER CLINICAL UNIT

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Maika González, Fátima Mercadillo, Mario Esteban Muñoz (since August)

OVERVIEW

Lynch syndrome is a very complex entity associated with high risks for a wide variety of malignancies, including colorectal, endometrial, ovarian, gastric, urinary tract, pancreatic, biliary, small intestinal, prostatic, and brain cancers. Until now, the malignancies developed in people with Lynch syndrome were treated exactly in the same way as their sporadic counterparts. However, recent therapeutic advances in the immunologic effects of microsatellite instability (MSI), the hallmark of Lynch syndrome associated tumours, have resulted in important changes in the treatment of these patients.

MSI, by definition, is characterised by the somatic accumulation of mutations, which subsequently produce potentially antigenic frameshift neopeptides that account for the infiltrating lymphocyte reaction classically observed in Lynch-associated tumours. The recent emergence of immune checkpoint inhibitors that work on the patients' own immune system has led to the use of this underlying biological characteristic to advance in the treatment of Lynch syndrome-associated tumours.

The Familial Cancer Clinical Unit (FCCU) is not only committed to screening blood samples with the aim of identifying germline mutations, but also to analysing tumour samples to determine their microsatellite status. Both findings play a critical role in the understanding of the molecular drivers of malignancy and the implementation of innovative precision-based therapies.

CLINICAL, DIAGNOSTIC AND RESEARCH HIGHLIGHTS

The clinical and diagnostic activities carried out by the FCCU through the consultancy in the Medical Oncology Department of Fuenlabrada's University Hospital, have contributed to the selection of patients who are good candidates for targeted therapies. In order to extend the study, we apply a multigene panel test to an increasingly larger number of pathologies. Ovarian cancer (OC) for instance, is a genetically heterogeneous malignancy that is potentially driven by multiple aberrant molecular pathways. Germline *BRCA1/2* mutations account for 65–85% of all hereditary OC, while mutations in Lynch genes (DNA mismatch repair genes) are responsible for 10–15% of these hereditary OC. Germline mutations drive the therapeutic strategy: OC associated to *BRCA1/2* mutations have a demonstrated sensitivity to PARP inhibitors, while immune checkpoint inhibitors are indicated for metastatic solid tumours associated with DNA mismatch repair deficiency.

Our clinical and diagnostic activities this year can be summarised as follows: 550 patients visited our consultancy at *HUF* (8.69% increase over 2017); and 508 genetic diagnostic studies were performed in the FCCU laboratory (18.69% increase). Among these studies, we identified 25 tumours with MSI, all of them potential candidates to be treated with monoclonal antibodies that target PD-1.

Our research in colorectal cancer (CRC) focuses on early-onset forms and multiple primary tumours. We recently reported the largest series of Synchronous Colorectal Cancers (SCRC), in which clonality was analysed by Single-Nucleotide Polymorphism array, and the subsequent statistical application; we were the first to correlate it with clinical phenotypes. Thirty-six per cent of our SCRC fulfilled clonality features. The existence of clonality within SCRC has important consequences throughout therapeutic management. The stratification in different categories may also serve as a starting point to more selectively analyse the molecular basis of SCRC and its relationship with environmental factors.

The FCCU also focuses its research efforts on less frequent cancer predisposition syndromes. One of these is the PTEN hamartoma tumour syndrome (PHTS), in which several aspects such as the high clinical heterogeneity usually result in a late diagnosis. We have studied this pathology at the clinical and molecular level in the largest series of Spanish patients with PHTS (145 probands). Overall, our findings are consistent with the syndrome descriptions in other populations, with a few exceptions such as a higher proportion of carriers of mutations in *PTEN* exon 1. We have also discussed the usefulness of the different diagnostic criteria proposed to date for this disease and have suggested recommendations based on our results. We are currently focusing on the search for phenotype modifiers, as in the case of the *KLLN* gene, as well as for other genetic factors that may explain the disease in *PTEN* wild type patients. For this last purpose, we are using a gene panel to look for mutations on the main pathway antagonised by PTEN – the PI3K/AKT/mTOR pathway – and are analysing whole exome sequencing data from selected cases. Our study continues to contribute to a better definition of PHTS and to help accelerate the diagnosis of the patients.

Addressing the functional consequence of germline missense variants involved in cancer genes is very important when prophylactic surgical removal of organs is the only therapeutic option to prevent the development of an aggressive cancer. In this context, we found 3 unrelated families with hereditary diffuse gastric cancer carrying the same germline missense variant in the *CDH1* gene: c.1679C>G. Through genetic and *in vitro* studies, we explored the effect of this variant and finally demonstrated its deleterious effect, suggesting that gastrectomy should be considered in patients harbouring this variant. ■

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MOLECULAR CYTOGENETICS AND GENOME EDITING UNIT

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'Titulado Superior (Advanced Degree)

OVERVIEW

Recurrent chromosomal rearrangements are very common and well-known hallmarks of cancer. One of their main consequences is the creation of new chimeric genes as a result of the fusion of the coding sequences of 2 different genes. The research activity of the Molecular Cytogenetics and Genome Editing Unit (MC&GEU) is focused on increasing the knowledge about the genetics of tumours and the discovery of new therapeutic targets. With the combined used of CRISPR genome editing and cytogenetic technologies, we are creating human *in vitro* models that recapitulate chromosomal, genetic and epigenetic cancer alterations. The goal of the Unit is to provide the CNIO and external researchers with the latest technologies used in the fields of molecular cytogenetics and genome editing. The Unit is constantly implementing and developing new technologies in the gene editing field. We also participate in collaborative projects with clinical and basic science investigators at the CNIO and other institutes.

"We have applied genome engineering approaches for cancer modelling, reproducing chromosome rearrangements and gene alterations. We provide access to the latest Cytogenetic and CRISPR technologies."

RESEARCH HIGHLIGHTS

Modelling cancer using CRISPR/Cas9 genome editing technology

Efficient methodologies for recreating cancer-associated chromosome aberrations and gene mutations are in high demand as tools for investigating how such events initiate cancer. We have recently demonstrated the feasibility of utilising gRNA/Cas9 ribonucleoprotein (RNP) complexes to model cancers driven by fusion genes generated by chromosomal rearrangements. We have optimised new strategies to enhance the efficiency of the CRISPR-mediated translocation induction in human stem cells, including mesenchymal and induced pluripotent stem cells. We found that the generation of targeted translocation is significantly increased by using a combination of ribonucleoprotein complexes (Cas9 protein+sgRNA) and ssODNs. The CRISPR-Cas9-mediated generation of targeted translocations in human stem cells opens up new avenues to model cancer.

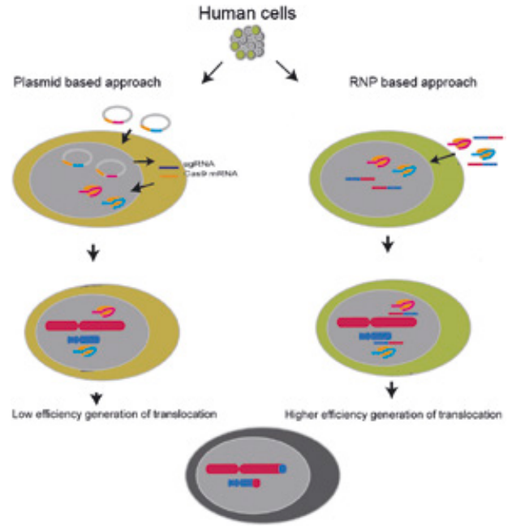

Figure Overview of efficient approaches for recreating cancer-associated chromosome translocations. Usage of RNP and ssODN efficiently recreates human chromosomal translocations.

Technological and translational activities

We provide state-of-the-art Molecular Cytogenetic and Genome Editing services. The Unit supplies research groups with various techniques that may provide more sensitive and accurate tools to analyse cancer cells, such as chromosome stability studies based on a combined array CGH-FISH approach, or the use of CRISPR libraries to perform high-throughput functional analysis. For gene editing experiments, we have set up a specific PCR-based FISH analysis to detect genome integration sites of small constructs including LV particles. As the field of cancer cytogenomics moves forward with the identification and cataloguing of recurrent chromosomal aberrations and gene mutations in a variety of human cancers, our CRISPR-based cellular platforms offer a rapid, precise and affordable opportunity to functionally interrogate the cancer genome. In 2018, we carried out over 1,500 assays for experimental and clinically-oriented projects. ■

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PATENT

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HUMAN GENOTYPING-*CEGEN* UNIT

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Titulado Superior (Advanced Degree)

OVERVIEW

The most abundant types of genetic variation are single nucleotide variants (SNVs) and copy number variants (CNVs). Association studies involving the large-scale analysis of both SNVs and CNVs in thousands of patients can help to identify genes underlying complex diseases such as cancer and drug responses. In this Unit we implement different high-throughput and cost-effective methods to measure from one to millions of SNVs and CNVs. In addition, epigenetic studies using whole-genome methylation arrays are performed in this Unit. Complementarily, research focused on the identification of predictive biomarkers for precision medicine is also undertaken.

"Matching cancer patients with treatments that are likely to be more effective and cause fewer side effects is what we strive for."

RESEARCH HIGHLIGHTS

Pharmacogenetic variants and response to neoadjuvant single-agent doxorubicin or docetaxel: a study in locally advanced breast cancer patients participating in the NCT00123929 phase 2 randomised trial. Docetaxel and anthracycline are widely used in the treatment of breast cancer despite the benefit being limited to a small proportion of patients, and preoperative biomarkers predictive of clinical outcome remain lacking. We carried out a pharmacogenetic study in 181 patients with locally advanced breast cancer who were previously enrolled in a phase 2 randomised clinical trial (NCT00123929), in which patients were randomly assigned to receive doxorubicin (anthracycline) or docetaxel (taxane) in neoadjuvance. We assessed whether genetic variants in 15 key transport or metabolism genes relevant to doxorubicin and docetaxel drugs could play a role as predictive biomarkers. We identified a genetic variant, located in the promoter of ABCC2, as having the strongest association with tumour response observed in patients treated with doxorubicin (P=0.009). We also identified a significant association for an intronic variant, located in CYP1B1, associated with docetaxel tumour response (P=2.15x10-4). Our integrated pathway-based approach allows revealing promising genetic biomarkers for treatment outcome in breast cancer patients (Ruiz-Pinto S *et al.*, 2018).

Genome-wide association study (GWAS) identifies three new loci associated with Ewing sarcoma susceptibility. Ewing sarcoma (EWS) is a paediatric cancer characterised by the EWSR1-FLI1 fusion. Our previous GWAS identified susceptibility loci at 1p36.22, 10q21 and 15q15. We performed a GWAS of 733 EWS cases and 1346 unaffected individuals of European ancestry. Our study replicates previously reported susceptibility loci at 1p36.22, 10q21.3 and 15q15.1, and identifies new loci at 6p25.1, 20p11.22 and 20p11.23. In the analyses of the new loci, there is evidence of informative eQTLs with nearby biologically plausible candidate genes that could be likely target genes for future functional investigations. It is remarkable that 6 independent susceptibility regions with relatively large effect sizes (estimated OR > 1.7) have been discovered in a sample of 733 EWS cases. In conclusion, our study provides support for a strong inherited genetic component to EWS risk and suggests that interactions between germline variation and somatically acquired EWSR1-FLI1 translocations are important etiologic contributors to EWS risk (Machiela MJ *et al.*, 2018).

New loci associated with risk to develop tobacco-induced lung cancer: genome-wide association study in heavy smokers. We genotyped 2.37 million SNPs across the genome in heavy smokers that either developed NSCLC at an early age (extreme cases), or did not present NSCLC at an advanced age (extreme controls), selected from a discovery set (n = 3631). We validated significant SNPs in 133 additional subjects with extreme phenotypes selected from databases including >39,000 individuals. Two SNPs were validated: rs12660420 (p combined = 5.66 × 10-5; OR combined = 2.80), mapping to a noncoding transcript exon of PDE10A; and rs6835978 (p combined = 1.02 × 10-4; OR combined = 2.57), an intronic variant in ATP10D. We assessed the relevance of both proteins in early-stage NSCLC. PDE10A and ATP10D mRNA expressions correlated with survival in 821 stage I-II NSCLC patients (p = 0.01 and p < 0.0001). PDE10A protein expression correlated with survival in 149 patients with stage I-II NSCLC (p = 0.002). In conclusion, we validated 2 novel variants associated with risk of developing tobacco-induced NSCLC in heavy smokers (Fusco JP *et al.*, 2018). ■

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