

Human Genetics Group



Javier Benítez

Group Leader

Javier Benítez obtained his PhD in Biology (Human Genetics) from the *Universidad Complutense de Madrid* in 1982. He completed postdoctoral studies at the Department of Physiopathology, University of Ulm (Germany), 1985; and then at the Department of Human Genetics, University of Munster (Germany), 1990; as well as at the Department of Ophthalmogenetics, University of Amsterdam (The Netherlands). He was appointed as Honorary Consultant at the Royal Marsden Hospital in London (UK), 2004, and as visiting scientist at Columbia University in New York, 2009.

Benítez spent several years working on the cytogenetics of human tumours at the *Fundación Jiménez Díaz*, Madrid, and from 1995 his group focused specifically on hereditary breast cancer and the characterisation of genes responsible for hereditary breast/ovarian cancer (*BRCA1* and *BRCA2*) and the search for new genes (*BRCAX*).

In 1997 he was appointed as Head of the Human Genetics Service at the *Fundación Jiménez Díaz*. He joined the CNIO in the year 2000 as Director of the Human Genetics Department and in 2005 was appointed Director of the Human Cancer Genetics Programme.

Over the past few years his research has focused on familial cancer in general as well as the search for low penetrance genes associated with cancer using high throughput technologies.

Benítez has been President of the Spanish Society of Human Genetics and is currently an active member of various scientific societies and reviewer for various scientific publications and funding agencies. He has been Professor of Human Genetics at the *Universidad Francisco de Victoria* in Madrid, and is Honorary Professor at the *Universidad Autónoma de Madrid*. He is also Director of the Spanish National Genotyping Centre (CEGEN, Madrid) since 2004.

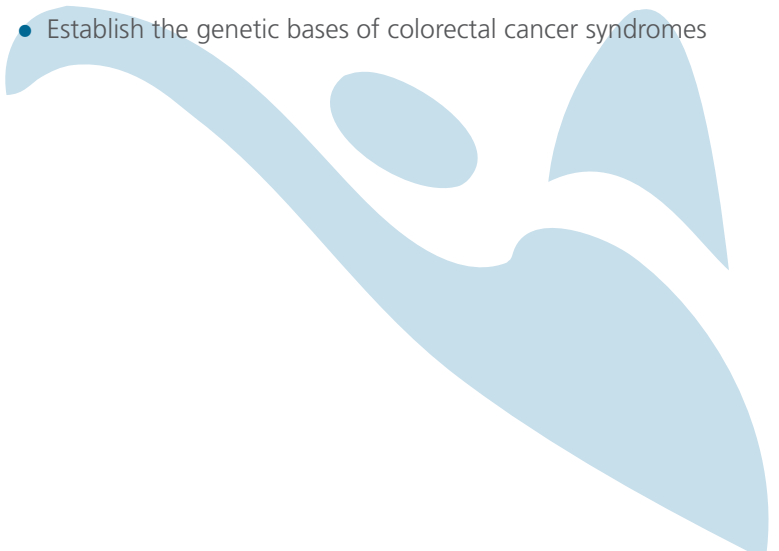
Summary

Our Group is interested in understanding the genetic bases of familial breast, ovarian and colorectal cancer, and to apply such knowledge to clinical practice through genetic counselling. Main activities include the location and identification of new susceptibility genes for both sporadic and familial cases, the identification of genetic and environmental modifier factors that can modulate cancer risk, and the design of biological experiments that can help achieve these objectives.

Our approaches range from disease to gene, from individual to population levels (genetic epidemiological studies) and from constitutional to somatic tissue – using classical genetic approaches such as linkage analysis and case control association studies, new high throughput technologies for genotyping and ultrasequencing of the whole genome or exome, the development of new bioinformatic tools for this research, and functional studies for data interpretation.

Strategic Goals

- To further insight into familial and sporadic breast cancer. Searching for new genes, modifier factors and genetic markers
- Molecular, immunohistochemical (IHC) and genomic characterisation of familial ovarian tumours
- Establish the genetic bases of colorectal cancer syndromes





Staff scientists: M. José García, Beatriz Martínez, Ana Osorio and Miguel Urioste. **Post-doctoral fellows:** Francisco J. Gracia, Iván Muñoz and Kira Yanowsky. **Graduate students:** Marta M. Kamieniak, Ricardo Ramires (until July), Bárbara Rivera, Eva Sánchez, Laura P. Saucedo, Miljana Tanic, Tereza Vaclova (since October) and Magdalena B. Zajac (until August). **Technicians:** Alicia Barroso, Samuel Domingo, M. Victoria Fernández, Fernando Fernández, M. Carmen González and Fátima Mercadillo.

Highlights

Breast cancer

Deciphering the genetic bases of familial breast cancer: over the past few years we have demonstrated that non BRCA1/BRCA2 tumours (BRCAX) are heterogeneous. Searching for BRCAX genes within the Fanconi Anaemia pathway we found that in addition to BRCA2 (*FANCD1*), there are two more genes – *FANCF* (*BRIP1*) and *FANCN* (*PALB2*) – whose mutations explain around 2-3% of BRCAX families; and one more new gene – *RAD51C* – which belongs to the DNA double-strand break repair pathway, responsible for 1-2% of breast and ovarian cancer families. We are currently involved in sequencing the whole exome of 9 families, of which at least 10 females are affected by breast cancer not associated with BRCA1/2 mutations, with the goal of finding more than one gene to explain these families.

Our search for low penetrance genes (LPGs) within the BCAC Consortium in collaboration with the CNIO Genotyping Core Unit and the Genetic Epidemiology Group has revealed 18 new LPGs, concluding that these new genes currently explain about 9% of familial breast cancer risk. These results together with data from other groups suggest that a high percentage of breast cancer families without a known aetiology still exist. Mammographic density is one of the possible risk factors for breast cancer. We have completed a genome-wide association study (GWAS) in families with extreme breast cancer phenotypes, in collaboration with M. Pollán from the *Centro Nacional de Epidemiología* (Madrid), to search for genes responsible for mammary density.

We are currently validating the results in an independent series.

Searching for modifier factors: regarding familial cases known to be associated with the high risk susceptibility genes *BRCA1* and *BRCA2*, we are participating in an international consortium to identify phenotypic modifiers of these genes: the CIMBA Consortium which has currently identified 10 modifier genes. The presence of these modifier genes in the genome of a BRCA carrier would modify the probability of developing breast and ovarian cancer from 40% to 85%. We are also coordinating a study on the genes involved in the Base Excision Repair Pathway (BER) as potential risk modifiers in a large set of 2000 BRCA1/2 carriers originating from the Spanish Consortium for the study of hereditary breast cancer.

Searching for genetic markers via expression arrays: our expression microarray study in familial breast cancer has demonstrated that BRCA1 tumours can be subclassified into two main groups according to their ER status. Regarding the BRCAX tumours we have shown that they are heterogeneous and can be divided into at least two main subgroups, classified as Luminal A and B. We have also defined a genetic signature associated with survival and drug response that seems to appear in all types of tumours.

MiRNA and familial cancer: we are defining the miRNA profiles associated with the genetic familial breast cancer subtypes – *BRCA1*, *BRCA2* and *BRCAX* – by analysing more than 100 of these familial tumours. The discovery

of miRNAs that differentiate these familial subtypes may improve diagnosis and provide new insight into the molecular biology of the tumours. In our analysis of miRNA expression in breast tumours and normal breast tissue, we have already identified a group of 30 miRNAs that are differentially expressed among these samples. More specifically, expression of these miRNAs was inhibited in the tumours. Computational analysis has predicted common pathways regulated by this group of miRNAs.

We have identified the MAPK signalling pathway as being significantly regulated by our set of miRNAs. Along these lines, inhibition of these miRNAs would result in a concomitant activation of MAPK signalling. Interestingly, the K-RAS gene is regulated by some of these miRNAs. Our *in vitro* studies in tumour cells expressing specific miRNAs have revealed an abnormal proliferation of the cells. We are currently validating the regulation of the K-RAS gene by miRNAs using luciferase assays (Figure 1).

The role of *TFDP1* and *CUL4A* genes in breast cancer development: using oligo array-based

comparative genomic hybridisation (aCGH) we have detected an amplicon in 13q that is present in 20% of both sporadic and familial basal tumours. Analysis of the genes located in the region showed that *TFDP1* and *CUL4A* were also overexpressed in the amplified cases.

We are currently analysing their possible oncogenic role by silencing (shRNA) and overexpressing (infection) these genes in several cell lines with different genetic backgrounds (Figure 2).

Ovarian tumours

Genomic and immunohistochemical (IHC) classification: a tissue microarray constructed with 75 familial and 125 sporadic tumours has been analysed with more than 30 antibodies from different signalling pathways, with the objective of classifying them from an IHC point of view and defining diagnostic, prognostic and drug response markers. Our results have shown several characteristics specific to the different subtypes and a signature (increased expression of ERCC1/PR) that correlated with survival and drug response. In parallel we have obtained

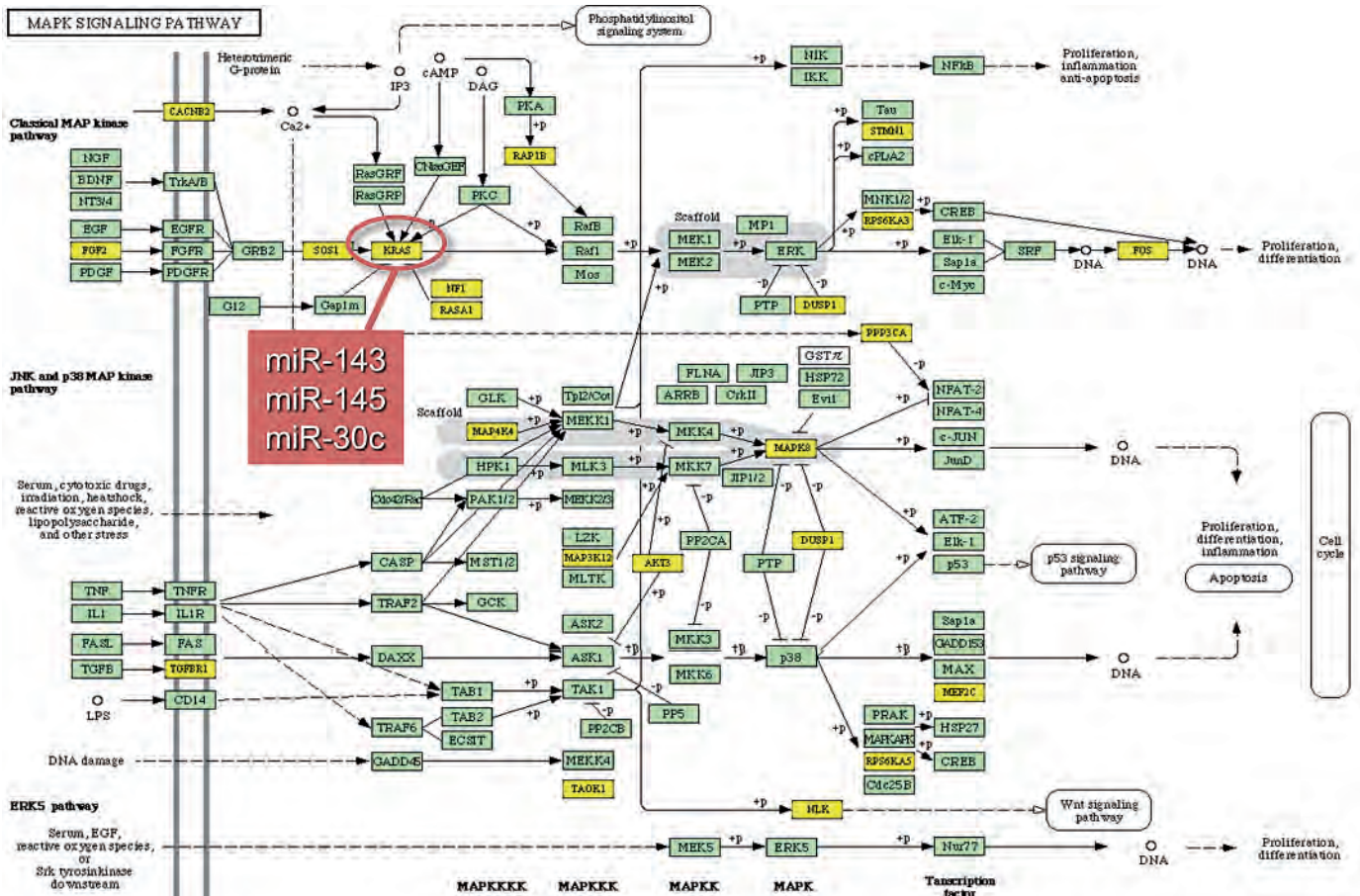


Figure 1: The MAPK signalling pathway depicting (in yellow) potential target genes of the 30 miRNAs differentially expressed in normal and tumour breast tissue samples. Interestingly, the K-RAS gene is regulated by three of these miRNAs.

data from high-resolution aCGH of the same cases and the next step will be to combine both IHC and genomic studies to generate a common pool of data that will facilitate a wide and integrated interpretation of results.

Familial colorectal cancer

We have defined the clinical and molecular characteristics in a series of Spanish families with classic Familial Adenomatous Polyposis (FAP). Studies showed that more than 20%

of the classic forms were apparently normal for both *APC* and *MUTYH* genes. A more thorough study of these negative families identified several uncommon *APC* inactivating alterations, such as allelic imbalance or 3'-end mutations. Since a proportion of these negative cases do not seem to be related with mutations in *APC/MUTYH* genes, we have recently embarked on a study to evaluate the involvement of other genes in the Wnt pathway.

Human Genetics service activity

Our group is a reference in Spain for genetic studies in familial cancer syndromes. In 2010 we carried out over 600 studies involving more than 25 genes responsible for different types of familial cancer syndromes. About 40% correspond to families with breast and ovarian cancer; 30% to colorectal cancer; 20% to endocrine cancer; and the remaining cases to rare genetic diseases.

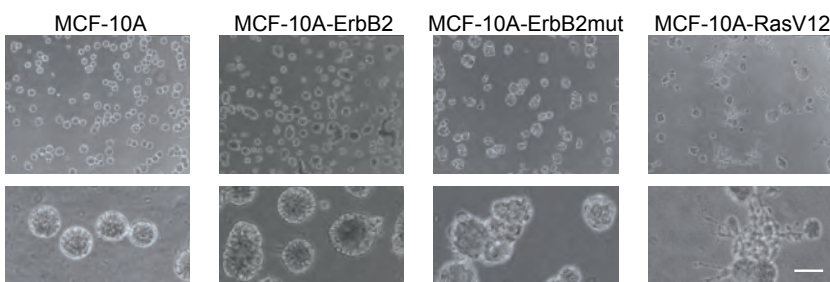


Figure 2: MCF10A cells with different backgrounds showing how a single genetic alteration can modify the phenotype of this cell line. Scale bar represents 100 μ m.

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