

Etienne Danis

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Education

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| PhD. in Biological and Medical Sciences | 2005 |
| Institute of Human Genetics, University of Sciences, Montpellier, France | |
| M.S. in Molecular Biology and Biochemistry | 2000 |
| University of Montpellier II, Montpellier, France | |
| B.S. in Molecular Biology and Biochemistry | 1999 |
| University of Montpellier II, Montpellier, France | |

Research Experience

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| Research Associate | 2013 –2016 |
| Pediatric Hematology/Oncology/BMT Department,
University of Colorado, Anschutz Medical Campus
Advisor: Dr. Tobias Neff | |

Identification of signaling pathways activated in novel murine models of acute lymphoblastic leukemia and acute myeloid leukemia

- ◆ Isolate, purify and cultivate murine hematopoietic stem and progenitor cells (HSPCs) from murine bone marrow
- ◆ Transduce HSPCs with viruses expressing oncogenes such NRAS, MLL-AF9 or MN-1
- ◆ Analyze microarray, RNA-seq and ChIP-seq data to identify critical signaling pathways
- ◆ Confirm the expression of genes associated with signaling pathways at the RNA and protein level
- ◆ Determine leukemic cell sensitivity to drugs targeting the newly identified signaling pathways
- ◆ Identify human leukemic cells exhibiting similar gene expression profiles and drug sensitivity compared to murine leukemic cells developed as potential models of acute lymphoblastic leukemia and acute myeloid leukemia
- ◆ Analyze histone modifications and transcription factor presence at gene promoters and enhancers using chromatin immunoprecipitation

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| Assistant Project Scientist | 2011 –2012 |
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Pharmacology Department, University of California, San Diego
Advisor: Dr. Jing Yang

Characterization of Twist1 and Twist2 involvement in liver injury and breast cancer metastasis

- ◆ Induce liver injury in wild-type and knock-out mice by intraperitoneally injections of tetrachloride
- ◆ Determine the expression of transcription factors and fibrotic genes in liver following tissue injury at the RNA level by reverse transcription (RT) followed by quantitative real-time PCR (qPCR) and at the protein level by immunoblotting (IB) and immunohistochemistry (IHC)
- ◆ Isolate, purify and culture primary hepatocytes, hepatic stellate cells and sinus endothelial cells from liver of wild-type and knock-out mice using liver perfusion, series of enzymatic digestions and Nycodenz gradient
- ◆ Follow the expression of transcription factors and fibrotic genes in cultured hepatic stellate cells after *in vitro* activation at the RNA level by RT-qPCR and at the protein level by immunofluorescence (IF)
- ◆ Determine the expression levels of diverse transcription factors and their target genes in murine and human mammary cancer cell lines following knock-downs using shRNA
- ◆ Develop phagocytosis assays using labeled apoptotic epithelial cells to test the effects of the different transcription factor knock-downs on phagocytic activity

Postdoctoral Fellow

2006 - 2011

Pharmacology Department, University of California, San Diego

Advisor: Dr. Jing Yang

Dissection of Twist1 transcriptional network in epithelial-mesenchymal transition and metastasis

- ◆ Induce the epithelial-mesenchymal transition (EMT) in immortalized human mammary epithelial cells (HMLE) using inducible Twist1-ER and Snai1-ER systems and using over-expression of Twist1 and Snai1
- ◆ Study the effects of diverse growth factors, secreted signaling proteins and signaling pathway inhibitors on the EMT process in murine and human mammary epithelial cells
- ◆ Identify early and late Twist1 and Snai1 target genes in HMLE Twist1-ER and HMLE Snai1-ER using Illumina microarrays
- ◆ Analyze microarray data
- ◆ Screen for Twist1-regulated transcription factors and signaling pathways critical for Twist1-induced EMT
- ◆ Design and generate lentiviral vectors expressing shRNAs against 25 different transcription factors including PRRX1, PRRX2, GLI1, GLI2, FOXF1, FOXF2, SIX2, RUNX2, ZEB1, ZEB2...
- ◆ Determine transcription factor target genes using the guilt-by-association approach
- ◆ Transfect cells to generate lentiviral particles used to infect normal and cancer cell lines
- ◆ Determine the knock-down efficiency of 150 shRNAs (individually tested) targeting the transcription factors by analyzing RNA and protein expression of transcription factors and their respective target genes
- ◆ Characterize their function on cell migration, cell invasion and interaction with the extracellular matrix

- ◆ Transform, fluorescently label and subcutaneously inject HMEC Twist1 cells with selected knock-downs in immunodeficient mice and monitor the formation of metastases using fluorescence microscopy

Postdoctoral Fellow

2005 – 2006

Gene Expression Laboratory, The Salk Institute for Biological Studies, La Jolla
 Advisor: Dr. Geoffrey Wahl

Isolation and characterization of mammary stem cells during murine embryonic development

- ◆ Design, maintain and characterize transgenic mice expressing fluorescently labeled histones under the control of a Wnt responsive promoter in order to follow and isolate murine embryonic mammary stem cells during embryogenesis
- ◆ Breed and genotype transgenic mice
- ◆ Isolate primary fibroblasts from murine embryos and adult mouse tails
- ◆ Isolate fluorescently labeled embryonic murine mammary buds (stage E14) and transplant into syngeneic mice
- ◆ Study Wnt pathway activation in murine mammary epithelial cell lines and in primary fibroblasts

Graduate Researcher

2000 – 2005

Genome Dynamics Laboratory, Institute of Human Genetics, Montpellier, France
 Advisor: Dr. Marcel Méchali

Role of transcription factors in the specification of DNA replication origins in *Xenopus laevis*

- ◆ Design, prepare and purify on cesium chloride gradients DNA vectors containing diverse transcription factor binding sites
- ◆ Micro-inject of DNA vectors in the presence of purified transcription factors (GAL4-VP16 and Sp1) in *Xenopus laevis* eggs in order to study the role of these transcription factors in targeting DNA replication initiation
- ◆ Extract and purify nascent single-stranded DNA using enzymatic treatment and sucrose gradients in RNase free conditions
- ◆ Analyze the distribution of nascent strand DNA by both competitive and real-time PCR
- ◆ Study of histone acetylation and deacetylation during chromatin reprogramming in early embryonic development of *Xenopus laevis* using specific inhibitors and IF techniques
- ◆ Develop helicase assays using Southern blotting techniques to analyze the processive helicase activity of purified MCM2-7 proteins
- ◆ Study transcription factor DNA binding affinity using electrophoretic mobility shift assay

Postgraduate Student Researcher

2000

Genome and Cancer Laboratory, Val d'Aurelle Cancer Research Center, Montpellier, France

Advisor: Dr. Pascale Cohen and Dr. Charles Theillet

Study of gene expression variations associated with tamoxifen resistance in MCF7 cancer cell lines

- ◆ Design and prepare cDNA mini-arrays on nylon membranes using 1000 single amplified PCR products corresponding to cDNA reported in the literature to be implicated in cancers
- ◆ Compare gene expression profiles between breast cancer cell lines resistant and sensitive to tamoxifen treatment

Postgraduate Student Researcher

2000

Genome Dynamics Laboratory, Institute of Human Genetics, Montpellier, France
Advisor: Dr. Domenico Maiorano and Dr. Marcel Méchali

Study of MCM proteins interaction with chromatin in *Xenopus laevis*

- ◆ In vitro synthesis of MCM2-7 proteins using rabbit reticulocyte extracts
- ◆ Purification of chromatin and immunodetection of DNA replication proteins at different time points during S phase

Undergraduate student

1999

Genome and Cancer Laboratory, Val d'Aurelle Cancer Research Center, Montpellier, France
Advisor: Dr. Paul Chuchana and Dr. Charles Theillet

Study of CpG islands differentially methylated in human breast cancer cell lines versus normal breast cell lines

- ◆ Purification of methylated CpG islands from fragmented genomic DNA isolated from human breast cancer cell lines and human normal breast cells using the DNA binding domain of MeCP2
- ◆ Cloning and DNA sequencing of purified CpG islands

Biology Skills

- ◆ Cell biology
Cell culture, transfection, transduction, proliferation assay, cell adhesion assay, scratch assay, transwell migration and invasion assays, collagen contraction assay, immunofluorescent staining, light and fluorescence microscopy, primary cell culture (fibroblasts, hepatocytes, hepatic stellate cells, sinus endothelial cells, hematopoietic stem cells), drug assays, flow cytometry, fluorescence-activated cell sorting (FACS)
- ◆ Biochemistry

Protein extraction, purification and quantification, protein expression using rabbit reticulocyte lysate, protein complex separation using sucrose gradients, immunoprecipitation, immunoblotting (including phospho-protein detection), immunofluorescence

◆ Molecular biology

Cloning, DNA and RNA isolation, purification and quantification, reverse transcription, sucrose and cesium chloride gradients, chromatin immunoprecipitation (ChIP), polymerase chain reaction (PCR), quantitative PCR (competitive and real-time), preparation of nylon mini arrays (macroarrays), Northern and Southern blottings, data analyses from mini arrays, microarrays (Illumina), next generation sequencing (RNA-seq, ChIP-seq)

◆ Histology

Masson's trichrome, carmine, red oil, hematoxylin and eosin staining, preparation and section of paraffin- and OCT-embedded tissues, immunohistochemistry

◆ Mouse handling

Breeding, plug checking, genotyping, retro-orbital bleeding, mouse surgery: anesthesia (isoflurane, ketamine/xylazine), subcutaneously/tail vein cell injections, mammary bud transplantation in cleared mammary fat pad, intraperitoneally drug injections, liver perfusion in order to isolate, purify and cultivate hepatocytes, hepatic stellate cells, endothelial cells, hematopoietic stem cells *in vitro*

◆ Bioinformatics

Microarray data analysis using Qlucore, GenomeStudio, GeneSifter, GeneSpring, OncoPrint and IPA softwares

Imaging using ImageJ, Spot, ImagePro softwares

DNA and protein analysis using Blast, Fasta, Clustalw, Repeat Masker, Promoter Scan, Transfac, TFsearch, ConTra, PromoSer, Whole Genome rVista, Fantom4, WebGestalt

Publications

◆ **Danis E**, Yamauchi T, Echanique K, Zhang X, Haladyna JN, Riedel SS, Zhu N, Xie H, Orkin SH, Armstrong SA, Bernt KM, Neff T. Ezh2 Controls an Early Hematopoietic Program and Growth and Survival Signaling in Early T Cell Precursor Acute Lymphoblastic Leukemia. *Cell Rep*. 2016 Mar 1;14(8):1953-65.

◆ **Danis E**, Yamauchi T, Echanique K, Haladyna J, Kalkur R, Riedel S, Zhu N, Xie H, Bernt KM, Orkin SH, Armstrong SA, Neff T. Inactivation of Eed impedes MLL-AF9-mediated leukemogenesis through Cdkn2a-dependent and Cdkn2a-independent mechanisms in a murine model. *Exp Hematol*. 2015 Nov;43(11):930-935

- ◆ Cayrou C, Grégoire D, Coulombe P, **Danis E**, Méchali M. Genome-scale identification of active DNA replication origins. *Methods*. 2012 Jun; 57(2):158-64
- ◆ Eckert MA, Lwin TM, Chang AT, Kim J, **Danis E**, Ohno-Machado L, Yang J. Twist1-induced invadopodia formation promotes tumor metastasis. *Cancer Cell* 2011 Mar 8; 19(3):372-86
- ◆ Stanojic S, Lemaitre JM, Brodolin K, **Danis E**, Mechali M. In *Xenopus* egg extracts, DNA replication initiates preferentially at or near asymmetric AT sequences. *Mol Cell Biol* 2008 Sep; 28(17):5265-74
- ◆ Lemaître JM, **Danis E**, Pasero P, Vassetzky Y, Méchali M. Mitotic remodeling of the replicon and chromosome structure. *Cell* 2005 Dec 2; 123(5):787-801
- ◆ Maiorano D, Cuvier O, **Danis E**, Méchali M. MCM8 is an MCM2-7-related protein that functions as a DNA helicase during replication elongation and not initiation. *Cell* 2005 Feb 11; 120(3):315-28
- ◆ **Danis E**, Brodolin K, Menut S, Maiorano D, Girard-Reydet C, and Méchali M. Specification of a DNA replication origin by a transcription complex. *Nat. Cell Biol.* 2004 Aug; 6(8): 721-30
- ◆ Vendrell JA, Magnino F, **Danis E**, Duchesne MJ, Pinloche S, Pons M, Birnbaum D, Nguyen C, Theillet C, Cohen PA. Estrogen regulation in human breast cancer cells of new downstream gene targets involved in estrogen metabolism, cell proliferation and cell transformation. *J. Mol. Endocrinol.* 2004 Apr; 32(2): 397-414

Communications

- ◆ **Oral presentation** – American Society of Hematology 56th Annual Meeting **2014**
 Danis E, Yamauchi T, Bernt K, Orkin S, Armstrong S, Neff T.
 Context Dependent Role of Polycomb Repressive Complex 2 in Acute Leukemia
- ◆ **Poster** - Era of Hope Conference, Orlando, Florida, USA **2011**
 Danis E, Danis C, Wolfe C, Yang J. Dissection of Twist1 transcriptional network in breast cancer metastasis
- ◆ **Poster** - Leading Innovative Networking and Knowledge Sharing conference, Vienna, Virginia, USA **2009, 2010, 2011**
 Danis E, Yang J. Dissection of Twist1 transcriptional network in breast cancer metastasis
- ◆ **Oral presentation** - DNA Replication and Genome Integrity, Salk Institute, La Jolla, California, USA **2004**
 Danis E, Menut S, Maiorano D, Vassetzky Y, and Méchali M. Specification of a DNA replication origin by transcription factors
- ◆ **Poster** - The 3R conference: Replication, Recombination and Repair, Presqu'île de Giens, France **2003**
 Danis E, Menut S, Girard-Reydet C, Maiorano D, and Méchali M. Specification of a replication origin by a transcriptional complex
- ◆ **Poster** - Harbor Laboratory, Cold Spring Harbor, New York **2001**
 Danis E, Brodolin K, Menut S, Maiorano D, Girard-Reydet C, and Méchali M. Specification of a DNA replication origin by the assembly of a transcriptional complex

Award and Scholarships

- ◆ Breast Cancer Research Program, Era of Hope Postdoctoral Award **2008 - 2011**
- ◆ George E. Hewitt Foundation for Medical Research Fellowship **2005 - 2006**
- ◆ French Ministry of Education Fellowship **2005 - 2004**
- ◆ French Association for Cancer Research Fellowship **2003 - 2004**
- ◆ French Ministry of Research Fellowship **2000 - 2003**

Teaching experience

- ◆ Training of undergraduate students, graduate students and postdocs in molecular biology, cell biology, biochemistry and mouse handling **2005 - 2016**
- ◆ Lecture instructor in molecular biology **2004 - 2005**
Graduate School of Biological Sciences, Montpellier, France
- ◆ Training of graduate students and postdocs in real-time PCR (experimental design, machine use, troubleshooting) using LightCycler 96 and 480 instrument from Roche, iCycler IQ from Biorad and 7500, 7900, QuantStudio5 systems from Applied Biosystems
University of Colorado, Denver, Anschutz Medical Campus **2013 - 2016**
University of California, San Diego **2006 - 2012**
Institute of Human Genetics, University of Sciences, Montpellier **2003 - 2005**

Activities

- ◆ Co-founder and co-CEO of the Academia Industry Alliance. Supervising team management (16-20 volunteers), communications, fundraising and marketing for networking events:
 - 3 major events (250-300 attendees, 35+ life science companies): 1st and 2nd annual Rocky Mountain BioTechnology Symposium in May 2015 and April 2016, 1st Life Science Industry Showcase in February 2016,
 - 1 monthly event (70-80 attendees, 20-30 life science companies): The Brews & Biotech Happy Hour at Cedar Creek Pub on the Anschutz Medical Campus
www.ucdenver.edu/aia and www.meetup.com/AIA-BBHH **2015-2016**
- ◆ Volunteer (programming, reviewing proposals and volunteer supervision) for the 10.10.10 networking event **2016**
- ◆ Co-organizer of the Institute of Human Genetics Symposium (300 attendees) **2001**
- ◆ Volunteer to visit cancer patients weekly at the Cancer Center of Val d'Aurelle in Montpellier with the Claude Pompidou Foundation **1997 - 1998**

Languages

English, French

Status

American Resident Alien (Green Card)