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Director of Research (DR2 – CNRS, section 22)
Project leader

Coordinator of "Stem cells and Cellular therapies" workpackage and of Scientific Animation and Communication at the Bordeaux Consortium of Regenerative Medecine (BxCRM).

Research Interests:

- Embryonic and induced pluripotent stem cells (ESC and iPSCs)
- Mechanisms of maintenance and exit of cell pluripotency/ differentiation/ apoptosis
- Impact of pleiotropic LIF cytokine, ECM and environmental paradigms on stem cell fates
- Cancer stem cells
- Regenerative medecine

Stem cells have the specific dual property to either self-renew or differentiate in specialized cells, depending upon environmental stimuli. The understanding of self- renewal mechanisms and of those triggering differentiation is of major importance for the control of stem cell plasticity and therefore for its potential application in cellular therapy. **Murine ES cells (mESC),** derived from the inner cell mass of blastocysts, are maintained pluripotent *in vitro*, in the naïve state, in the presence of LIF (Leukemia Inhibitory Factor). This cytokine, from the IL6 family, displays pleiotropic effects depending upon cell types and cell maturity. LIF is conserved in the non-eutherian vertebrate species, and has evolved as being the "nidation hormone" in mammals.

During this last decade, my work was dedicated to the identification and the understanding of targets of LIF involved in the maintenance of mESC pluripotency and in differentiation processes. In addition, we have investigated the mechanisms of pluripotency/differentiation cell switches and characterized plasticity windows in which cells could revert a committed to a more immature fate in a LIF-dependent way. Environmental parameters (like oxygen concentration or regulators of the Extra Cellular Matrix) have also been investigated in these processes. Our work highlights the fact that genetic programs activated in pluripotent cells grown under physioxic concentration of O2 (3%, which mimics O2 concentration in embryos) are different than those operating under 20% O2, the

hyperoxic condition more classically used for cell culture and in which, so far, most transcriptome and proteome analyses have been performed. We are further exploring these new genetic programs at work under physioxia.

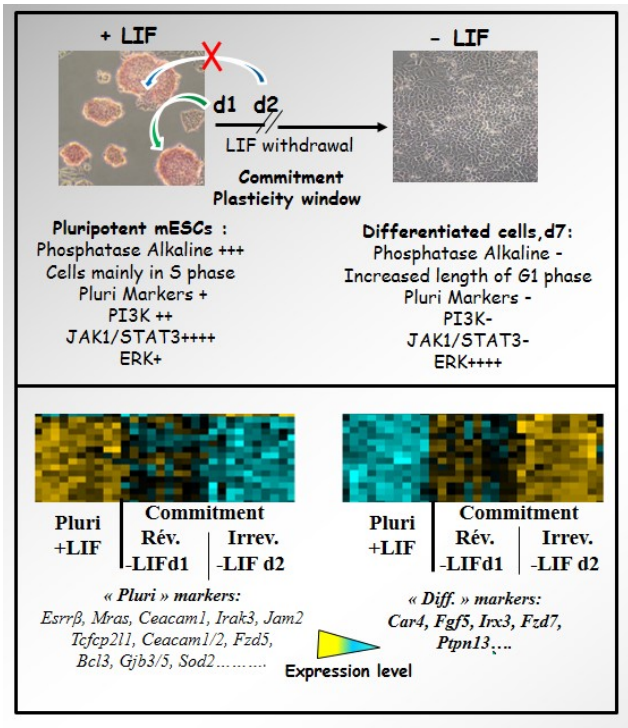
In addition, it is well known that ESCs are in metastable states and that gene expression is heterogeneous among clusters of pluripotent cells grown *in vitro*. This feature is relevant to the "pepper" and "salt" gene expression which has been observed for pluripotent master gene (like Nanog) in the early blastocysts. We are currently investigating the mechanisms of heterogeneity of mESCs. In addition, in this cell model, we have also studied, the apoptotic crisis of ES-derived differentiated cells (which occurs within 3 to 4 days upon LIF withdrawal) and find relevant links between apoptosis and differentiation.

We have also investigated the impact of LIF in **cancer stem cells** and have characterized expression profiles of LIF targets and of "stemness" genes in a collection of **glioma-derived cancer stem cells**, obtained under collaboration with the laboratory of MP Junier/H. Chneiweiss,U1130 Inserm, Neuroscience Paris Seine, UPMC and in **gastric cancer-derived stem cells** (in collaboration with Dr. C. Varon and C. Staedel, University of Bordeaux). In addition we have set up a "stemness sensor test" which allows to evaluate the impact of the tumor-derived conditioned medium to maintain pluripotency of mESC cells. **Our aim** is now to evaluate whether this test could help to score tumor specimen with a "stemness index" helping to better make a diagnosis and a relevant treatment. In that context we are also characterizing the link of LIF with the Hippo pathway, recently shown to display anti-metastatic effect in breast cancer.

In the BioTis INSERM unit that I have recently joined (January 2016), I will develop new approaches to study human induced pluripotent stem cells (iPSCs) and their potentialities to form bone-like organoids. With the skills of the BioTis laboratory around Biomaterials, Bioprinting and the development of new scaffolds for stem cells (as acellular human amniotic membrane), I will investigate various ways to build an efficient model towards bone repair.

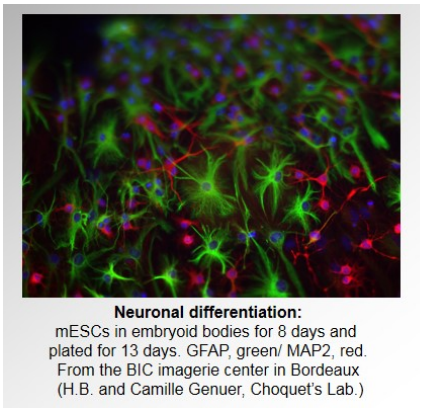
Keywords/expertise:

- Embryonic Stem cells (ESC and iPSC)
- Cancer stem cells (glioma and gastric cancer)
- Pluripotency
- Cell plasticity
- Reprogrammation
- Differentiation
- Neurons
- Apoptosis
- LIF/gp130 signaling
- MAPK/ PI3K signaling
- Hypoxia/ Physioxia
- Extracellular matrix
- Regenerative Medicine
- Cell-based therapies
- Translational medicine



The mESC model : To study mESCs and their derivatives in pluripotent, committed and differentiated states, by molecular, biochemical and functionnal approaches.

Cell cultures labelled with AP kit (which reveals alcaline phosphatase activity by a pink color) and transcriptomic tree view representations of gene expression are shown in the upper and lower parts of the figure respectively.



Neuronal differentiation:
mESCs in embryoid bodies for 8 days and plated for 13 days. GFAP, green/ MAP2, red.
From the BIC imagerie center in Bordeaux
(H.B. and Camille Genuer, Choquet's Lab.)

An example of **ES-derived** neuron (MAP2+) and glial cells (GFAP+) obtained after a simple differentiation procedure with mESCs . Around 50% of cell culture are neuronal/glial-like cells.

Selected publications:

1-Trouillas, M., C. Saucourt, B. Guillotin, X. Gauthereau, L. Ding, F. Buchholz, M.X. Doss, A. Sachinidis, J. Hescheler, O. Hummel, N. Huebner, R. Kolde, J. Vilo, H. Schultz, and H. Boeuf (2009) Three LIF-dependent signatures and gene clusters with atypical expression profiles, identified by transcriptome studies in mouse ES cells and early derivatives. *BMC Genomics.* 10(1): 73-84.

2-Trouillas M, Saucourt C, Guillotin B, Gauthereau X, Taupin JL, Moreau JF and Boeuf H. (2009) The LIF cytokine : towards adulthood . *European Cytokine Network,* 20, 51-62.

3-Schulz, H., Kolde, R., Adler, P., Aksoy, I., Anastassiadis, K., Bader, M., Billon, N., Boeuf, H., et al, and A. K. Hatzopoulos (2009) The FunGenES database: a genomics ressource for mouse embryonic stem cell differentiation. *PloS ONE*. 4, e6804. 1-14.

4-Guitard, A., Debeissat, C., Hermitte, F., Villacreces, A., Ivanovic, Z., Boeuf, H. and V. Praloran (2011) Very low oxygen concentration (0.1%) reveals two functionally distinct

subpopulations within the FDCP-mix hematopoietic cell line. *Cell Death and Differentiation*, 18(1):174-82.

5-Mathieu M.E., Saucourt C., Mournetas V., Gauthereau X., Thézé N., Praloran V., Thiébaud P and H. Bœuf (2012) LIF-dependent signaling: new pieces in the Lego. *Stem cell Reviews and Reports,* Mar;8(1):1-15.

6-Mathieu, ME, Faucheux, C., Saucourt, C., Soulet, F., Gauthereau, X., Fédou, S., Trouillas, M., Thézé, N., Thiébaud, P. and H. Boeuf (2013) Mras GTPase is a novel stemness marker that impacts mouse embryonic stem cell plasticity and Xenopus embryonic cell fate. *Development*, 140, 3311-3322.

7-Zeineddine D., Abou-Hammoud A., Mortada M. and H. Bœuf (2014) The oct4 protein: more than a magic stemness marker. *Am J Stem Cells*, 3 (2), 74-82.

8-Hammoud AA, Kirstein N, Mournetas V, Darracq A, Broc S, Blanchard C, Zeinedine D, Mortada M and H. Boeuf (2016) Murine Embryonic Stem Cell Plasticity Is Regulated through Klf5 and Maintained by Metalloproteinase MMP1 and Hypoxia. *PloS ONE*. 11(1): e0146281.

9-Nguyen P.H., Giraud J., Staedel C., Chambonnier L., Dubus P., Chevret E., Bœuf H., Gauthereau X., Rousseau B., Fevre M., Soubeyran I., Belleannée G., Evrard S., Collet D., Mégraud F. an C. (2016) All-trans retinoic acid targets cancer stem cells in gastric carcinoma and inhibits patient derived gastric carcinoma tumor growth. *Oncogene. In Press.*

10-H. Boeuf et al. (2016) Stemness activities correlated with high expression level of *Klf5* and *Tead4* in glioblastoma cancer cell lines derived from patients. *In Prep.*

Teaching Activities:

- UE stem cells, Master BCPP, Bordeaux (4 to 8h/ year)
- UE Biotherapies, for the Physicians, Bordeaux (2 h/ year)
- Intervention in Lycées of Bordeaux for Pre-graduate students

Clinical Activities:

Fundings:

Subvention ARC, 2009-2010 : 50 000 Euros
Subvention FR Transbiomed, 2011-2012 : 12 000 Euros
Contrat Région Aquitaine, 2013-2015, Equipment : 68 000 Euros
Préciput ANR, 2013, Equipment : 35 000 Euros
INCA, 2015-2017, Consommables and salary : 67 000 Euros

Education:

May 2000: Habilitation à Diriger des Recherches, University of Strasbourg, France

1989-1992: Post-doctoral internship, Professor H. Varmus, University of California, San Francisco, San Francisco, U.S.A.

1987-1988 CNRS position, Research associate

1983-1987: PhD, University of Strasbourg, Professor Claude Kedinger, IGBMC laboratory, Strasbourg, France

1978-1982 : Engineer in Biology, Genetics, Biochemistry: C.U.S.T./ POLYTECH, University Blaise Pascal, Clermont-Ferrand, France

Links:

ReaserchGate: https://www.researchgate.net/profile/Helene_Boeuf/stats/reads?date=2016-02-21
BxCRM: Bordeaux Consortium for Regenerative Medecine: **https://bcrm.u-bordeaux.fr/**