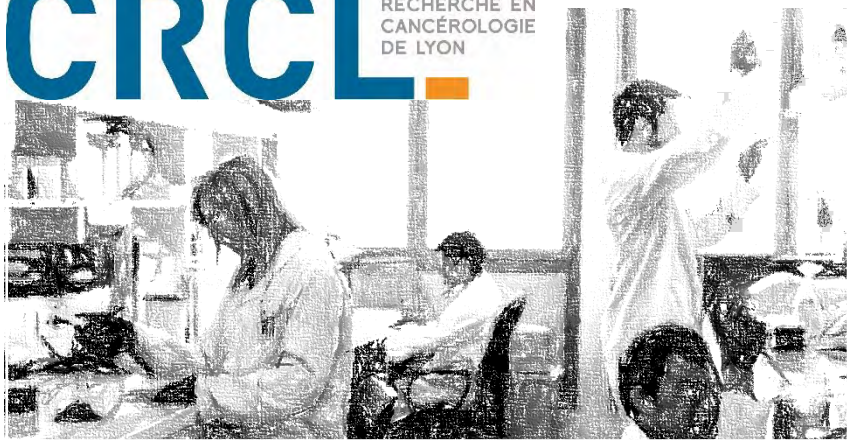
CRCL CENTRE DE RECHERCHE EN CANCÉROLOGIE DE LYON



CRCL Newsletter N°30

March – April, 2020

Hello Everyone,

The health crisis caused by Covid19 has changed our professional and personal life in recent weeks. First of all, I hope you are all well, as well as your loved ones, and thank you for all of your engagement, your adaptability during this new situation, and your patience. Thank you all, for your immediate response in setting up the PCA, for adapting to working from home, and also for participating in transversal missions for CRCL or to support the activity of CLB.

May 11 has been announced as the date for gradual deconfinement. Although everyone is impatient to resume work, we must coordinate, in conjunction with your team leaders, to organize this return in best conditions to ensure your safety and respect the distancing measures, which will continue to apply. Your team leaders have already prepared and organized for the return to activity plan. We have also opened the Cheney D reception room since April 27 to allow for receiving of packages, an activity essential for a gradual resumption of research activities.

Some information about our Hcéres evaluation: while it was scheduled for mid-March, the Hcéres visit obviously could not be organized. The evaluation still took place, and was based on the file, as well as on questions that were addressed to the director of CRCL and to each of the 22 applicant teams. In addition, the CRCL director was also interviewed on March 27, and together with Charles Dumontet, we defended the achievements of the Center on the last contract and on our project for the next 5 years. In addition, specific questions were addressed to the research staff and teacher-researchers, ITA, doctoral students and post-docs. Following this evaluation, we received on May 1, the preliminary report from Hcéres. This very positive report demonstrates the position taken today by the CRCL in the French research landscape and this allows us to defend the various postulating teams of the CRCL with Inserm and CNRS in June.

The coming weeks will probably still be binding; we will still have to be patient and kind. I count on the involvement of you all and I thank you in advance.

Take good care of yourself and your loved ones,
Patrick Mehlen.

Team presentation : « BMP, Ecosystem, Stemness and Dynamic in Cancer », Dir V. Maguer-Satta

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Platform presentation : Research Pathology Platform

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CRCL's latest publications

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TEAM PRESENTATION

Presentation of the «BMP: ecosystem, stemness & dynamic in cancer» team.

The "BMP: ecosystem, stemness & dynamic in cancer" team is led by Véronique Maguer-Satta (Research Director at the CNRS), and is located on the 4th floor of the Cheney D building.

The emergence of this team, originally called "BMP, tumoral niche and resistance", dates back to the arrival of Véronique Maguer-Satta on the Centre Léon Bérard (CLB) site, in 1998. At that time, she integrated the unit directed by Jean-Pierre Magaud (Inserm U453), within the team of Ruth Rimokh as research beneficiary of the CLB.

The objective of her recruitment, instigated by Thierry Philip (former Director of the CLB) and Eric Archimbaud (Professor/Hospital practitioner in Hematology at the Civil Hospices of Lyon HCL, Edouard Herriot), was to develop a novel research topic revolving around hematopoiesis, stem cells and transformation.

A group is thus created around this topic that will subsequently be housed by Charles Dumontet's team, prior to being integrated within the team co-directed by Jean-Yves Blay and Christophe Caux at the creation of the CRCL in 2011. The group then emerged as a team recognized by the CRCL in 2013, and validated by the AERES (or current HCERES) in 2015.

Based on the awareness that eradication of Cancer Stem Cells (CSCs) is of utmost importance, owing to their role in treatment resistance and cancer relapse, the "BMP: ecosystem, stemness & dynamic in cancer" team focuses on the implication of BMP (Bone Morphogenetic Proteins) pathways (members of the TGF-β family) in regulating stem cells (SCs) and CSCs. Their workforce is in particular directed at characterizing the role of the BMP pathway in orchestrating the tumoral microenvironment, by analyzing the constant dialogue between CSCs and their micro- and macro- environment (including biomechanical parameters) during tumor initiation and treatment resistance.

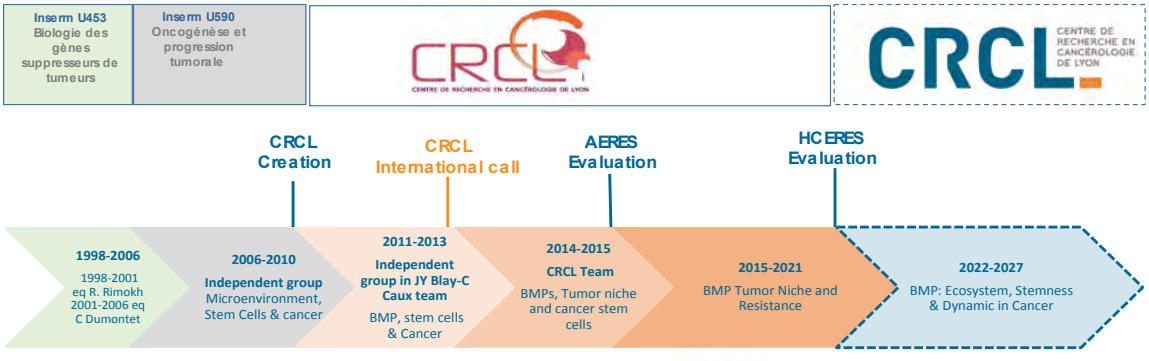
Studies conducted in the last few years unveiled dysregulations of the BMP signaling pathway that could be detected at diagnosis in human hematopoietic and mammary gland tissues, both in CSCs and in their microenvironment (niche). Their findings demonstrated the implication of dysregulations in the emergence and expansion of CSCs by the aberrant amplification of a natural process. Their results highlighted the role of BMP2 and BMP4 proteins in controlling SCs and the constant dialogue with their microenvironment, as well as tumorigenesis and treatment resistance, in addition to their well-known effects at a later stage of transformation.

Beyond their scientific discoveries, some aspects of this team's research have a societal impact. Indeed, they demonstrated potentially pro-tumorigenic effects of bisphenol S, with levels close to those obtained with bisphenol A or stress. This finding questions to some extent the definition of endocrine disruptors. Such results could provide key elements to improve our understanding of the impact of environmental pollutants on health and drive preventive measures, while clarifying the legal definition of endocrine dysfunctions.

At the time of its creation, the team included, aside from its leader VéroniqueMaguer-Satta (DR1 CNRS), a permanent engineer (Thibault Voetzel, IR1 Inserm), a technician (Sandrine Jeanpierre, CLB) and two professors/hospital practitioners of the CLB (Emmanuel Delayand Franck Nicolini). It has since gained considerable workforce with the arrival of Boris Guyot (CRCN CNRS, in 2015), of Dr Amine Belhabri and of the professor emeritus Mauricette Michallet (professors/hospital practitioners at the CLB in 2015 and 2019, respectively), of Sylvain Lefort (post-doc, in 2017) and of Eve-Isabelle Pécheur (CRCN CNRS, in 2019). Their expertise is complemented by that of 4 PhD students (Nora Jung, Kawtar Arizkane, Léa Barral and Emma Risson) and of an assistant engineer (Kevin Geistlich). Finally, the team often welcomes internships from different medical and scientific backgrounds, from France or abroad, and is highly attractive to Erasmus students. The team is currently housing two Masters students, one with medical training and the other via an Erasmus program (Simon Aho, Cristina Cuella-Martin).

Based on this workforce, the team is currently studying the effects of BMPs on early transforming events using in-house designed tools, such as human bone marrow cell lines and a standardized 3D model. This latter will enable them to investigate the efficacy of novel drugs or therapeutic strategies *in situ*, for instance to eradicate CSCs in leukemia or to decipher mechanisms underlying quiescence in bone metastases. The team is also implicated in integrated and multidisciplinary studies in biomechanics, with the aim of analyzing BMPs and their signaling as mechanoreceptors during transformation and SC persistence, as well as during treatment response. Finally, a novel biophysics tool was recently developed via a collaboration, to study biomechanical signaling, in particular that transduced via the BMP pathway during cell confinement. The overall objective being to identify early markers of patients at a higher risk of developing cancer, as well as to design innovative tools and strategies to detect and prevent tumor growth and escape mechanisms.

In addition to their research activities, several members of the team actively take part in teaching duties in certain Masters courses, locally and at the national level, as well as in training students with technical backgrounds, a majority of whom pursue a scientific career. Members of the team are also implicated in a variety of events to disseminate knowledge to a large audience (The march for research organized by the non-profit organization Déchaîne ton cœur), in public lectures/conferences ("Ma Thèse en 180 secondes, welcoming school pupils, Ligue contre le cancer, Comité féminin de dépistage du cancer du sein 74, Fondation de France …), in developing a harmonious environment at the CRCL (strong implication in working groups and internal events). The team also favors the synergistic actions of CRCL researchers and CLB practitioners, as it currently houses 4 practitioners, and takes part in joint working groups such as the Centre's IT Committee (Comité Informatique d'Établissement), the Environmental Committee and the Ethics Think Tank.



Presentation of the Research Pathology Platform East

The Research Pathology Platform East is located on the 3rd floor of the Cheney B building, under the direct supervision of Nicolas Gadot (operational management) and Pr SylvieLantuejoul (scientific coordinator). The platform was presented alongside the CRCL at the latest Hcéres evaluation for the 2021-2025 contract.

The **Research Pathology Platform East** (RP) was created in 2016 following the integration on the Centre Léon Bérardsite of the Anipath platform , formerly located at the Laennec Faculty of Medicine and directed by Pr JY Scoazec and N Gadot.

The mission of the platform is to facilitate collaborations between researchers and pathologists of the CLB, the HCL CHU Lyon-Est and VetAgroSup working on fundamental, pre-clinical and translational research projects, requiring histopathological expertise to analyze human or animal tissues. The RP platform has so far collaborated with 17 teams of the CRCL. Each year, over 5,000 murine samples and 1,700 human tumors are processed and analyzed in the framework of research projects in oncology.

To make these collaborations fruitful, the team of the RP platform draws on the strong expertise of their scientific coordinator Pr.Sylvie Lantuejoul, of their operational and technical manager, as well as of three technicians: Julie Valantin, expert in immunohistochemical (IHC) techniques and *in situ* hybridization (ISH), Laura Francols, expert in standard anatomical pathology techniques and Quality standards, and Clémentine Le Nevé, expert in multiplex immunofluorescence (IF) and NanoString (DSP), under the supervision for the latter state-of-the-art technological platform of Francesca Damiola, PhD. A clinical project coordinator, Céline Altun, also works part-time on the platform.

S LANTUEJOUL N GADOT F DAMIOLA J VALANTIN

C LE NEVE L FRANCOLS C ALTUN

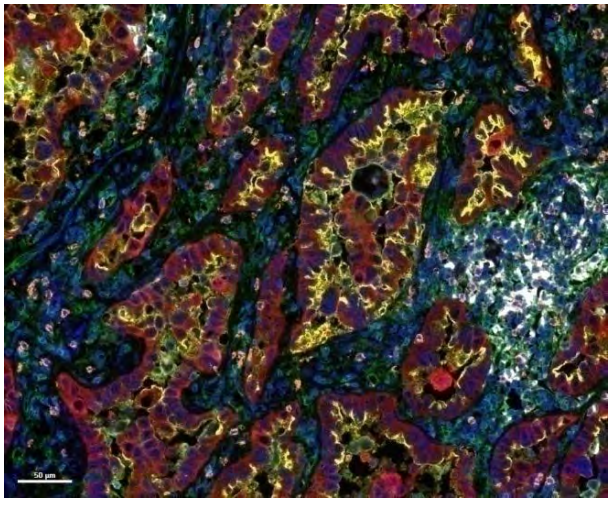
From the onset, the platform was supported by the Synergy Lyon Cancer Foundation to acquire the equipment necessary to conduct all standard histopathological techniques, as well as IHC and ISH. A laser microdissection microscope was financed by the "Investissementd'avenirPhénocan" program. Finally, the call for proposals IRICE financially supported by the Auvergne Rhône Alpes region,enabled the platform to purchase a multiplex IHC processing platform. See framed text for further details on the different techniques/technologies available.

- **Anatomical pathology technique** to characterize the morphology of tumor tissues or cells derived from humans or animal models, either frozen or fixed, using standard or custom staining (completely automated processing platform for dehydration Leica ASP6025, paraffin embedding, microtomography and staining Leica ST5010-CV5030)
- **Automated immunohistochemical/immunocytochemical techniques**, chromogenic or fluorescent staining, *In Situ*Hybridization (ISH): Automated Roche Discovery XT, Roche Benchmark ULTRA, Leica Bond RX
- **Multiplex immunohistochemical technique** following the acquisition of the Vectra Polaris Akoya platform in 2019
- **Digital pathology platform** including a brightfield and fluorescent 3D HISTECHslide scanner
- **Image analysis platforms** (n=3) equipped with the Halo Indica labs and Inform software
- **Production of TMA** (Tissue MicroArray) using the Tissue microarrayers (ExciloneMinicore)
- **Macro/microdissection** using the PALM Laser UV (Laser MicrodissectorLeica LMD6)
- **Digital Spatial Profiling** (DSP) Nanostringtechnology acquired in 2020

Innovative technologies offered by th PR platform

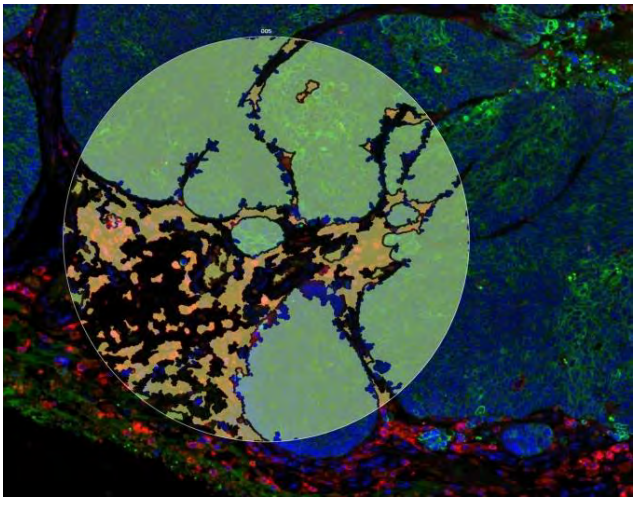
The Vectra Polaris Akoya Platform:

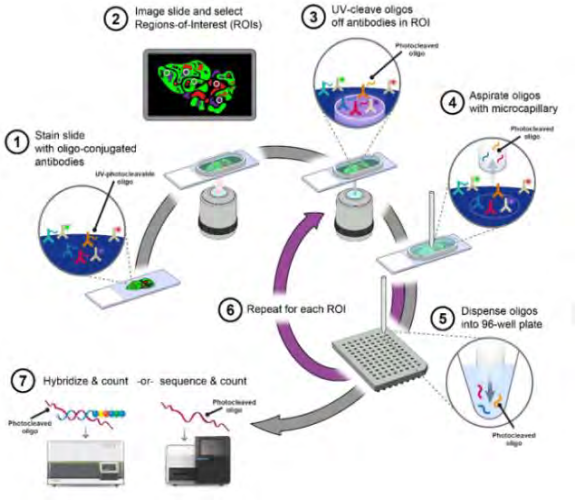
This platform can be used to perform 6-8 multiplex fluorescence immunolabeling on a single FFPE (Formalin-Fixed Paraffin-Embedded)tissue section, based on a sequential IHC methodology that adds tyramide-fluorophore signals. This methodology relies on a complete master of standard IHC techniques and prevents issues linked with cross reactions between species. The Vectra Polaris scanner acquires images on up to 9 fluorescent channels. Spectrum quantification and marker analysis can then be conducted using the Inform or Halo software.



The GeoMx Digital Spatial Profiling (DSP) NanoString Platform:

This innovative technology provides a spatial quantification of over 50 proteins or up to 1,830 RNAs on a single FFPE tissue section of pre-identified regions of interest ranging from several cells to 600 µm in diameter. This technique combines: 1- morphological and IHC approaches to determine the regions of interest, 2- an IHC- or ISH-like technology for the hybridization of antibodies or of oligonucleotide-tagged probes, 3- a quantification step of these oligonucleotides to obtain a specific read-out of the abundance of each protein or RNA.





Conditions for gaining access to the platform and the services provided/project monitoring:

If necessary the platform can provide technical assistance for a particular step of your project, or can manage your project entirely. Our collaboration with researchers is a key aspect of our work and should be instigated at the onset and followed throughout the project. For studies using patient cohorts, the involvement of a pathologist is crucial, for patient selection, marker validation, and project follow-up. Of note, it is very important to comply with the rules and regulations of the RGPD (General Rules and Regulations for Data Protection).

From a practical point of view, you should contact the platform via e-mail anapath.recherche@lyon.unicancer.fr at the beginning of your project and send them a document summarizing your project. The microdissection and image analysis platforms are open to users following a training period and by complying with the booking system. All of the information you may need to submit a project, as well as the protocols used are available at *V:\DRTI Plateformes\FormulairesAnapath Recherche* (CLB network only).

CRCL PUBLICATIONS

Netrin-1 promotes naive pluripotency through Neo1 and Unc5b co-regulation of Wnt and MAPK signalling

Aurélia Huyghe, Giacomo Furlan, Duygu Ozmadenci, Christina Galonska, Jocelyn Charlton, Xavier Gaume, Noémie Combémorel, Christina Riemenschneider, Nicolas Allègre, Jenny Zhang, Pauline Wajda, Nicolas Rama, Pauline Vieugué, Isabelle Durand, Marie Brevet, Nicolas Gadot, Thomas Imhof, Bradley J. Merrill, Manuel Koch, Patrick Mehlen, Claire Chazaud, Alexander Meissner & Fabrice Lavial

Nature Cell Biology, **22**, pages389–400(2020)

https://doi.org/10.1038/s41556-020-0483-2

This publication was the subject of a press release from the Léon Bérard Center
See here

Gene-specific actions of transcriptional coregulators facilitate physiological plasticity: Evidence for a physiological coregulator code

Michael R.Stallcup, Coralie Poulard

Trends in Biochemical Sciences, In press

https://doi.org/10.1016/j.tibs.2020.02.006

Intracellular Cytidine Deaminase Regulates Gemcitabine Metabolism in Pancreatic Cancer Cell Lines

Bjånes TK, Jordheim LP, Schjøtt J, Kamceva T, Cros-Perrial E, Langer A, Ruiz de Garibay G, Kotopoulis S, McCormack E, Riedel B.

Drug Metab Dispos. 2020 Mar;48(3):153-158.

https://doi.org/10.1124/dmd.119.089334

ATF4-Dependent *NRF2* transcriptional regulation promotes antioxidant protection during endoplasmic reticulum stress

Sarcinelli C, Dragic H, Piecyk M, Barbet V, Duret C, Barthelaix A, Ferraro-Peyret C, Fauvre J, Renno T, Chaveroux C, Manié SN

https://doi.org/10.3390/cancers12030569

SRF-FOXO1 and SRF-NCOA1 Fusion Genes Delineate a Distinctive Subset of Well-differentiated Rhabdomyosarcoma

Karanian M, Pissaloux D, Gomez-Brouchet A, Chevenet C, Le Loarer F, Fernandez C, Minard V, Corradini N, Castex MP, Duc-Gallet A, Blay JY, Tirode F.

Am J Surg Pathol. 2020 Mar 16

https://doi.org/10.1097/PAS.0000000000001464

Netrin-1 and Its Receptor DCC Are Causally Implicated in Melanoma Progression

Boussouar A, Tortereau A, Manceau A, Paradisi A, Gadot N, Vial J, Neves D, Larue L, Battistella M, Leboeuf C, Lebbé C, Janin A, Mehlen P.

Cancer Res. 2020 Feb 15;80(4):747-756

https://doi.org/10.1158/0008-5472.CAN-18-1590

Generation of an Fsp1 (fibroblast-specific protein 1)-Flpo transgenic mouse strain

Cardot-Ruffino V, Chauvet V, Caligaris C, Bertrand-Chapel A, Chuvin N, Pommier RM, Valcourt U, Vincent D, Martel S, Aires S, Kaniewski B, Dubus P, Cassier P, Sentis S, Bartholin L

Genesis. 2020 Mar 19:e23359

https://doi.org/10.1002/dvg.23359

We created a transgenic mouse strain ([Fsp1-Flpo]) expressing the Flpo recombinase in fibroblasts. The [Fsp1-Flpo] strain represents a genuine tool to further target the recombination of transgenes with Frt sites specifically in cells of mesenchymal origin or with a fibroblastic phenotype.

Generation of a conditional Flpo/FRT mouse model expressing constitutively active TGFβ in fibroblasts

Cardot-Ruffino V, Chauvet V, Caligaris C, Bertrand-Chapel A, Chuvin N, Pommier RM, Valcourt U, Vincent DF, Martel S, Aires S, Kaniewski B, Dubus P, Cassier P, Sentis S, Bartholin L.

Sci Rep. 2020 Mar 3;10(1):3880

https://doi.org/10.1038/s41598-020-60272-3

In order to analyze the effects of increased microenvironmental TGFβ concentration *in vivo*, we developed an original conditional transgenic mouse model (Flpo/Frt system) expressing bioactive TGFβ in fibroblasts, a cell population present in the microenvironment of almost all tissues.

Comprehensive molecular and pathological evaluation of transitional mesothelioma assisted by deep learning approach: a multi institutional study of the International Mesothelioma Panel from MESOPATH Reference Center

Journal of Thoracic Oncology

Salle FG, Le Stang N, Tirode F, Courtiol P, Nicholson AG, Tsao MS, Tazelaar HD, Churg A, Dacic S, Roggli V, Pissaloux D, Maussion C, Moarii M, Beasley MB, Begueret H, Chapel DB, Copin MC, Gibbs AR, Klebe S, Lantuejoul S, Nabeshima K, Vignaud JM, Attanoos R, Brcic L, Capron F, Chirieac LR, Damiola F, Sequeiros R, Cazes A, Damotte D, Foulet A, Giusiano-Courcambeck S, Hiroshima K, Hofman V, Husain AN, Kerr K, Marchevsky A, Paindavoine S, Picquenot JM, Rouquette I, Sagan C, Sauter J, Thivolet F, Brevet M, Rouvier P, Travis WD, Planchard G, Weynand B, Clozel T, Wainrib G, Fernandez-Cuesta L, Pairon JC, Rusch V, Girard N.

J Thorac Oncol. 2020 Mar 9

https://doi.org/10.1016/j.jtho.2020.01.025

High levels of plasma interleukin-17A are associated with severe neurological sequelae in Langerhans cell histiocytosis.

Ismail MB, Åkefeldt SO, Lourda M, Gavhed D, Aricò M, Henter JI, Delprat C, Valentin H.

Cytokine. 2020 Feb;126:154877

https://doi.org/10.1016/j.cyto.2019.154877

Hepatitis B Core-Related Antigen: An Alternative to Hepatitis B Virus DNA to Assess Treatment Eligibility in Africa

Clinical Infectious Diseases

Shimakawa Y, Ndow G, Njie R, Njai HF, Takahashi K, Akbar SMF, Cohen D, Nayagam S, Jeng A, Ceesay A, Sanneh B, Baldeh I, Imaizumi M, Moriyama K, Aoyagi K, D'Alessandro U, Mishiro S, Chemin I, Mendy M, Thursz MR, Lemoine M.

Clin Infect Dis. 2020 Mar 17;70(7):1442-1452

https://doi.org/10.1093/cid/ciz412

The purpose of this study is to find markers of HBV replication as surrogates of viral load, difficult and expensive to set up in low/middle income countries; This may help improving HBV diagnosis in highly endemic areas and patient's linkage to care.

Full-length 5'RACE identifies all major HBV transcripts in HBV-infected hepatocytes and patient serum.

JOURNAL OF HEPATOLOGY

Stadelmayer B, Diederichs A, Chapus F, Rivoire M, Neveu G, Alam A, Fraisse L, Carter K, Testoni B, Zoulim F.

J Hepatol. 2020 Feb 20. pii: S0168-8278(20)30102-1

https://doi.org/10.1016/j.jhep.2020.01.028

Guidance for design and endpoints of clinical trials in chronic hepatitis B - Report from the 2019 EASL-AASLD HBV Treatment Endpoints Conference

Cornberg M, Lok AS, Terrault NA, Zoulim F

J Hepatol. 2020 Mar;72(3):539-557

https://doi.org/10.1016/j.jhep.2019.11.003

JOURNAL OF HEPATOLOGY

Targeting p53 and histone methyltransferase restores exhausted CD8+ T cells in HCV infection

Barili V, Fisicaro P, Montanini B, Acerbi G, Filippi A, Forleo G, Romualdi C, Ferracin M, Guerrieri F, Pedrazzi G, Boni C, Rossi M, Vecchi A, Penna A, Zecca A, Mori C, Orlandini A, Negri E, Pesci M, Massari M, Missale G, Levrero M, Ottonello S, Ferrari C

Nat Commun. 2020 Jan 30;11(1):604

https://doi.org/10.1038/s41467-019-14137-7

nature communications

Hepatitis C virus (HCV) infection spontaneously resolves in 20–40% of the cases whereas in 60–80% of infected patients the virus is not cleared, and the infection becomes chronic. The different disease outcomes can be identified at early stages of infection making hepatitis C an ideal model to study the process of T cells differentiation from early exhaustion-committed to late fully exhausted CD8+ T cells in humans. In this work, the result of a long lasting and ongoing collaboration with the immunology team lead by Carlo Ferrari at the Parma University in Italy, we show that in early HCV infection, exhaustion-committed virus specific CD8+ T cells display a marked upregulation of transcription associated with impaired glycolytic and mitochondrial functions, that are linked to enhanced ataxia-telangiectasia mutated (ATM) and to p53 signalling. After evolution to chronic infection, exhaustion of HCV-specific T cell responses is instead characterized by a broad gene downregulation associated with a wide metabolic and anti-viral function impairment, which can be rescued by inhibitors of the EZH2 and the G9a histone methyltransferase (HMT). These results suggest that HMTs/p53 inhibitors might be valuable not only for treatment of HCV positive patients not responding to last-generation antivirals, but also for other chronic pathologies characterized by chronic inflammation and associated with T cell dysfunction, including cancers and not restricted to virus-related hepatocellular carcinoma.

Hepatitis B protein HBx binds the DLEU2 lncRNA to sustain cccDNA and host cancer-related gene transcription

Salerno D, Chiodo L, Alfano V, Floriot O, Cottone G, Paturel A, Pallocca M, Plissonnier ML, Jeddari S, Belloni L, Zeisel M, Levrero M, Guerrieri F

Gut. 2020 Feb 29.

https://doi.org/10.1136/gutjnl-2019-319637

Gut

Hepatocellular carcinoma (HCC) represents the fourth leading and fastest rising cause of cancer death (841,000 new cases and 782,000 deaths annually; GLOBOCAN: http://globocan.iarc.fr) and hepatitis B virus (HBV), with 250 million people chronically infected at risk to develop HCC, accounts for >50% of the cases. Here we show that: a) the viral protein HBx binds the promoter region, increases its transcription and induces the accumulation of the DLEU2 lncRNA in infected hepatocytes; b) nuclear lncRNA DLEU2 directly binds HBx and the histone methyltransferase enhancer of zeste homolog 2 (EZH2), the catalytic active subunit of the polycomb repressor complex 2 (PRC2) complex; c) HBx and DLEU2 co-recruitment on the cccDNA displaces EZH2 from the viral chromatin to boost transcription and viral replication; d) DLEU2-HBx association with target host promoters re-wires EZH2 repressive function to favor the non-canonical transcriptional activation of a subset of cancer related EZH2/PRC2 target genes in HBV-infected cells and HBV-related HCCs. Our results highlight the ability of HBx to bind host lncRNAs to impact on the epigenetic control of both viral cccDNA and host genes and provide a new key to understand the role of DLEU2 and EZH2 overexpression in HBV-related HCCs and HBx contribution to hepatocytes transformation.

Publishing director: Patrick Mehlen
Rédaction : Pierre Chaumont - Blandine Bruneel / Translation from French : Brigitte Manship – Quynh Chuong