DOCTORAT BIOLOGIE BRETAGNE SANTE LOIRE

Year 2022

THESIS TOPIC

Subject N° (to be completed by the ED):	**FUNDING:** x **Requested** ☐ **Acquired**	**Funding origin: INSERM/ARED**
Thesis title: RNA exosome-mediated processing and pathophysiology of plasma cell differentiation		3 keywords: RNA exosome, Plasma cell differentiation, multiple myeloma
Unit / team: : UMR 1236, équipe Bigres		
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Socio-economic and scientific context (approximately 10 lines):

A new field of biology has recently been opened by the discovery of essential regulatory functions for long-ignored molecules, the non-coding RNAs. These act in particular in the maintenance of the integrity of the genome structure and the regulation of its expression, and their catabolism is therefore also of major importance. The RNA exosome is a protein complex involved in the processing and degradation of different RNA classes. B lymphocytes are the actors of humoral adaptive immunity, with a terminal differentiation into immunoglobulin (antibody) secreting plasma cells. During B cell maturation, many non-coding RNAs are produced and their regulation by the RNA exosome is crucial. It has been shown that in the absence of this complex B cells have a defect in antibody production, with alterations in antigen affinity (during somatic hypermutation or SHM), and a drastic decrease in "switch" recombination (class recombination or CSR) aimed at diversifying antibody classes for an optimal immune response (Laffleur et al., Nature Genetics, 2021).

Beyond these mechanisms, the ability of the RNA exosome to govern the terminal differentiation of B cells into plasmablasts (PBs) and plasma cells (PCs) has not been explored. PCs have a unique transcriptional program, with approximately 6-fold higher RNA production than B cells, which likely requires careful RNA processing and monitoring. Moreover, the DIS3 subunit of the RNA exosome is frequently mutated in multiple myeloma, an aggressive and lethal cancer of PCs, but the contribution of these mutations to the initiation and/or progression of the pathology and resistance to treatment remains unknown.

The aim of this thesis is therefore to explore the pathways of sRNA surveillance during human B cell activation and plasma cell differentiation, and in their pathological counterpart, multiple myeloma.

Working hypothesis and aims (approximately 8 lines):

The contribution of the RNA exosome to human physiology is poorly established, while pathologies are caused by mutations affecting its subunits (Laffleur et al, Trends in Cell Biology, 2019). The DIS3 catalytic subunit is frequently mutated in hematologic malignancies, and a correlation has been established between DIS3 mutations and immunoglobulin gene translocations in multiple myeloma (MM). MM is a highly aggressive malignancy of PCs with a poor prognosis and the second most common hematological cancer. Myeloma cells are highly heterogeneous at the genetic level and show significant genomic aberrations. The contribution of DIS3 loss-of-function mutations to the disease remains unknown.

We hypothesize that RNA surveillance is a gatekeeper of genomic stability, and that mutations in the DIS3 gene then participate in the initiation and/or progression of MM.

The role of DIS3 in the physiological differentiation of PCs and its involvement in MM will therefore be investigated.

Main milestones of the thesis (approximately 12 lines):

Axis 1. RNA exosome and plasma cell differentiation.

The impact of DIS3 on the physiological differentiation of PCs will be studied using CRISPR/Cas9 genome editing and an in vitro differentiation system of PCs from human B cells. The role of DIS3 on differentiation will be assessed by flow cytometry and on the transcriptional program of B/PB/PC cells by qPCR and RNA-seq. This will reveal the coding and non-coding transcriptomes of these populations and potentially identify the co-factors involved in RNA processing/degradation during PC differentiation, and the epitranscriptomic mechanisms involved. The accumulation of chromatin-associated RNAs could modify the accessibility of DNA to DNA-binding transcription factors, this hypothesis will be tested by ChIP (chromatin immunoprecipitation).

Axis 2. RNA surveillance and genomic instability in multiple myeloma

In order to explore the mechanisms regulating genome stability following the loss of function of DIS3 we will study primary differentiating cells, lymphoma and MM lines where DIS3 is inactivated. The impact of DIS3 on the accumulation of non-coding RNAs and R-loops will be assessed by RT-qPCR, RNA-seq, and DRIP (R-loop immunoprecipitation). Mutagenic agents or AID could access this DNA and induce mutations, which will be evaluated by sequencing. The interaction between non-coding RNA accumulations and chromatin changes related to genomic instability will be tested directly by ChIP. The susceptibility of DIS3 mutant MM cells to undergo translocations will be assessed by LAM-HTGTS. We expect more translocations in DIS3 deficiency, a bias in DNA repair, and the presence of insertions/deletions will be quantified. Genome organization will be studied by 3C-HTGTS, which allows high resolution. Finally, cancer aggressiveness in DIS3 mutants can be assessed directly in vivo by transplanting myeloma cells into immunodeficient mice.

Scientific and technical skills required by the candidate (2 lines):

- Solid knowledge in immunology and cancerology

- Competence in molecular biology, high-throughput sequencing and cell biology

3 publications from the team related to the topic (last 5 years):

Laffleur B, Lim J, Zhang W, Chen Y, Pefanis E, Bizarro J, Batista CR, Wu L, Economides AN, Wang J, Basu U.“Noncoding RNA processing by DIS3 regulates chromosomal architecture and somatic hypermutation in B cells.” 2021, **Nature Genetics**

Nair L*, Zhang W*, **Laffleur B***, Jha MK*, Lim J, Lee H, Wu L, Alvarez NS, Liu ZP, Munteanu EL, Swayne T, Hanna JH, Ding L, Rothschild G, Basu U., “Mechanism of noncoding RNA-associated N6-methyladenosine recognition by an RNA processing complex during IgH DNA recombination.” 2021, **Molecular Cell**

Lim J, Giri PK, Kazadi D, **Laffleur B**, Zhang W, Grinstein V, Pefanis E, Brown LM, Ladewig E, Martin O, Chen Y, Rabadan R, Boyer F, Rothschild G, Cogné M, Pinaud E, Deng H, Basu U. 2017, **Cell**

National and international collaborations:

National: Jérôme Moreaux (IGH, Montpelliers), Laurent Delpy et Sandrine Le Noir (Université de Limoges), Charles Dumontet (CRCL, Lyon), Hervé Avet-Loiseau (IUCT-Oncopole, Toulouse), Bertrand Séraphin (IGBMC, Strasbourg), Amin Khamlichi (IPBS, Toulouse)

International: Uttiya Basu (Columbia University, New York), Junghyun Lim (Jeonbuk National University, Korea)