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Titre de la thèse : *« Identification et caractérisation des gènes régulateurs épigénétiques jouant un rôle « moteur » dans le cancer du sein »*

Résumé

Breast cancer (BC) is the most common cancer type and leading cause of cancer death in women worldwide. BC can be stratified into molecular subtypes varying in phenotype, gene expression and prognosis according to the expression of hormone receptors, and is caused by a combination of environmental, genetic, and epigenetic factors contributing to its development. Efforts in sequencing cancer genomes revealed that epigenetic regulator genes (ERGs), a group of over 400 genes involved in gene expression regulation through different mechanisms, are frequently mutated in human cancers. In a recent work, our group confirmed and extended this finding by revealing high rates of ERG mutations, copy number and expression alterations across cancers, including in BC, highlighting the involvement of ERG alterations in cancer hallmarks and the need to functionally validate driver roles of disrupted ERGs. Due to its key role in cell identity control, epigenetic deregulation may contribute to different cancer-associated phenotypes, such as the emergence of cancer stem cells (CSCs) characterized by high plasticity and the ability to form and maintain tumors, representing an important challenge in overcoming aggressive cancer phenotypes including metastasis, tumor recurrence and therapy resistance. However, specific epigenetic drivers of BCs and breast CSCs have not been characterized in depth.

In this thesis, we aimed to (i) identify ERG alterations with driver potential across BCs and in specific BC subtypes, (ii) identify putative epigenetic drivers ("epidrivers") of (cancer) cell plasticity and the acquisition of breast CSC-like characteristics, and (iii) functionally characterize the role of at least one candidate epidriver. To this end, we first applied an *in-silico* screening approach searching for the most frequent ERG alterations in BC tumors. We identified top ERGs with driver potential based on frequent genetic and expression aberrations across BCs and in specific subtypes, and ERG deregulations with an

impact on clinical outcome. We next applied a comprehensive *in-vitro* loss-of-function CRISPR/Cas9 approach targeting 426 ERGs in non-tumorigenic mammary cells, leading to the identification of several histone modifiers that may be involved in the acquisition of breast CSC-like markers and cell plasticity. Disruption in one of the top candidate genes, the histone deubiquitinase *BAP1*, was further validated for the acquisition of breast CSC marker expression in three-dimensional mammary spheroid models and extensively characterized at the transcriptome, epigenome, and phenotypic levels, revealing striking changes in gene expression and chromatin accessibility impacting cell cycle, cellular organization, membrane structure, and glycosylation pathways. We further aimed to unravel BAP1's role in BC progression using a model of aggressive BC, and preliminary results showed that *BAP1* disruption led to enrichment in breast CSC markers and transcriptional deregulation of several oncogenic pathways, impacting tumorigenesis and aggressive phenotypes. Finally, we optimized and applied different tools for further validation and characterization of putative BC epidrivers, including a CRISPR activation approach for the assessment of ERG amplification/overexpression in mammary and BC cell models, and the generation and genetic disruption of organoid and tumoroid models derived from breast tissue samples, that will allow candidate epidriver characterization in *ex-vivo* models.

The work presented here expands the knowledge on frequent epigenetic deregulations in BC and its associated subtypes and unveils key candidate epidrivers associated with worse clinical outcomes and the acquisition of cell plasticity and stemness markers in breast cells, one of which we validated and characterized. Together, these findings may constitute a strong basis for biomarker discovery and therapeutic targeting of epigenetic mechanisms contributing to BC-associated phenotypes.