Blueprints of disease: precision platforms for modelling breast cancer

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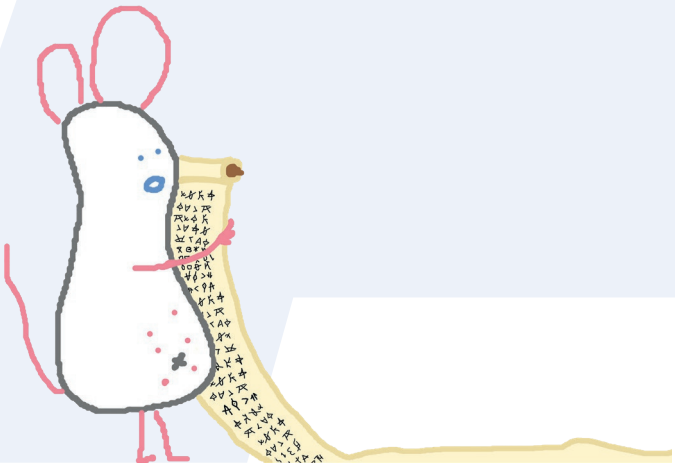

APPENDICES

English Summary
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ENGLISH SUMMARY

Preclinical models are useful and necessary tools in cancer research; they aid in understanding tumour biology, testing therapeutic strategies, and translating discoveries into the clinic. Over the past decades, a plethora of breast cancer (BC) models ranging from immortalised cell lines, organoid systems, cell line-derived xenografts (CDXs), patient-derived xenografts (PDXs), genetically engineered mouse models (GEMMs), and (mostly carcinogen-induced) rat models, has fuelled key advances in our understanding of breast cancer initiation, progression, and therapy response. Each of these systems has provided unique insights, yet none can fully capture the complexity of human disease, where hormone signalling, tumour heterogeneity, immune interactions, and evolutionary dynamics shape clinical behaviour. As the field continues to progress, the demand for models that are both biologically accurate and experimentally versatile has grown sharper, with their translational power increasingly judged by how well they reflect patient-relevant phenotypes and inform therapeutic innovation. Within this evolving landscape, luminal breast cancers present a particular challenge as they constitute the majority of BC cases worldwide, rely heavily on endocrine dynamics, and yet remain underrepresented in robust preclinical platforms.

Chapter 2 lays the conceptual foundation of this thesis by mapping the molecular and clinical complexity of luminal BC. It highlights the distinction between luminal A and B tumours, the contrast to non-hormone-dependent BC, their distinct mutational and proliferative features, and the clinical challenge posed by variable response to endocrine therapy and late relapse. This chapter describes all available model systems for luminal BC and highlights their strengths, weaknesses and the latest advancements in the respective field. It also establishes the critical need for modelling platforms that can recapitulate endocrine biology and genotype-phenotype relationships with sufficient resolution to inform translational research.

Chapter 3 introduces the rationale for using rats as a species and why they may be better suited to model luminal BC than mice. Rat mammary gland architecture, hormone physiology, and tumour biology more closely resembles that of humans and thereby offers advantages by maintaining natural hormone levels in a fully immune-competent setting, and with opportunities for longitudinal sampling. All rat models developed for luminal BC over the last decade are classified and their advantages, limitations and

applications are discussed thoroughly. Establishing the rat as a preclinical host sets the stage for subsequent platform innovations.

Building on the journey of rat models in the previous chapter, **chapter 4** presents the SMART (somatically modified autochthonous rat tumour) model platform, a cornerstone of this thesis. By delivering oncogenic drivers such as $PIK3CA^{H1047R}$, MYC, and CCND1 directly into the mammary ductal epithelium of immunocompetent rats, SMART models faithfully mirror the initiation and evolution of luminal tumours. The system enables systematic dissection of how individual or combined mutations influence tumour initiation, ER dependence, immune microenvironments, and therapy resistance. Crucially, SMART demonstrates that genotype-specific phenotypes can be captured and directly aligned with human datasets, underscoring its translational relevance.

Chapter 5 utilises the somatic modelling strategy described in the previous chapter and advances the technological aspect further by integrating precision gene editing. Using cytosine base editors, this work establishes proof-of-principle for introducing clinically relevant point mutations *in vivo* with high efficiency, low toxicity, and preserved tissue context. This approach moves beyond descriptive modelling to functional interrogation of specific variants, offering a scalable path to test the oncogenicity or therapeutic relevance of patient-observed mutations. This chapter aims to extend the scope of somatic modelling to nucleotide-resolution genetic alterations, paving the way for a new generation of high-fidelity, mutation-specific BC models.

Chapter 6 complements these somatic approaches with large-scale phenotypic and transcriptomic characterisation of orthotopic cell line-derived xenograft (CDX) models. By assembling an extensive panel of BC CDXs, spanning all BC subtypes, this work demonstrates how scale, rather than singular models, can yield insights into conserved transcriptional programmes driving morphology and aggressiveness beyond subtype classifications. In particular, TGF-β signalling emerges as a central pathway linking morphology to aggressiveness, an insight that would have remained hidden without systematic, large-scale comparison.

Chapter 7 aims to integrate the different strands described in the previous chapters into a platform-based modelling philosophy. Rather than relying on singular models with narrow applicability, this thesis advocates for modular,

multi-platform approaches where somatic (rat) models, *in situ* gene editing, and CDXs each play complementary roles. Together, they enable cross-validation, mechanistic depth, and translational anchoring in a way no single system can achieve.

Taken together, the work presented here advances BC modelling on several fronts: establishing the rat as a superior host for ER+ tumour studies, introducing the SMART system for autochthonous somatic modelling, pioneering *in situ* base editing for precision functional genomics, and scaling CDX characterisation to uncover emergent biological programmes. By generating and interlinking these platforms, this thesis not only overcomes longstanding limitations of traditional models but also provides a blueprint for translationally relevant preclinical research. Looking forward, the convergence of somatic engineering, advanced gene editing, and multi-model integration holds the promise of not only refining our understanding of luminal BC biology, but also driving the development of personalised therapeutic strategies for patients.