Tailoring the tools to study prostate cancer metastasis

La Manna, F.

Annexes

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Nederlandse samenvatting
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Summary

The last century witnessed an unprecedented acceleration in the rate of discoveries and innovations in almost all fields of knowledge, including medicine. From antibiotics to the structure of DNA, from recombinant proteins to neuroscience, we managed to understand and to address a multitude of conditions and diseases that were afflicting mankind for centuries if not millennia. Owing to our technical advances, complex diseases like cancer progressively shifted from being underdiagnosed and often lethal to being increasingly more understood and manageable. Some cancer types however, including prostate cancer, proved more challenging to investigate, requiring a multidisciplinary joint effort, as well as the development of more refined tools, to unfold.

The work included in this thesis fits in this context and aimed at developing novel tools to advance our understanding of advanced prostate cancer.

In **Chapter 1** the current landscape of prostate cancer is introduced, keeping a patient-oriented perspective and illustrating the most challenging aspects of this disease: its multifocality and clonal heterogeneity, the stratification of patients into risk groups and the associated therapeutic approaches. The most updated and acknowledged studies on prostate cancer genetics are reported and commented, distinguishing between the alterations associated with early-stage prostate cancer from those most frequently found in the advanced disease. A specific focus is then dedicated to the currently available research tools for the investigation of prostate cancer, ranging from *in vitro* models like cell lines and organoids to patient-derived xenografts (PDX) and genetically engineered mouse models.

The yet unaddressed problem of metastatic prostate cancer is then reviewed in **Chapter 2**, covering in particular the bone metastatic disease. Here, the mechanisms of bone metastasis formation are discussed, including the current models of metastatic dissemination, homing, dormancy and pathological reactivation. A concise section on the experimental models of bone metastasis an of the clinical management of the metastatic disease closes the review.

The experimental work presented in **Chapter 3** moves from clinical specimens of bone metastatic breast and prostate cancer, identifying the mTOR pathway as highly relevant for the latter. The molecular findings were further validated in an organoid-based drug screening of bone metastatic prostate cancer. A molecular investigation is then carried out to assess the effects of mTOR blockade on clonal dynamics in two different PDX models of bone metastatic prostate cancer. A novel mTOR-targeting drug is then investigated and

compared to other mTOR-targeting drugs *in vitro*, *ex vivo* and *in vivo*. The conclusions of this work endorsed the further investigation of this pharmaceutical strategy, supporting its pathway towards a clinical use for prostate cancer.

In **Chapter 4** a novel PDX model of early-metastatic, microsatellite-instable prostate cancer is presented. Microsatellite instability is an uncommon feature in this disease and identifies cancers with a distinct natural history. The developed PDX represents a valuable tool for the study of this subgroup of prostate cancer. This novel model, together with two additional prostate cancer PDX models was then implemented into a mid-throughput, organoid-based drug screening for drugs already approved for the treatment of cancers other than prostate. A shortlist of effective drugs was generated and used to develop a near-patient drug screening, using organoids from patients' biopsies. A relevant point shown in the study was the high correspondence of the organoid models used with the original tissue, thereby representing a viable translational tool for prostate cancer research.

The focus of **Chapter 5** was dedicated to an often-overlooked topic: the development of a structured methodology for literature data mining. The review illustrates the roles of Cripto, an oncofetal protein involved in the regulation of multiple physiological and pathological processes, including cancer initiation and progression. A keyword-based method was developed and used to build a conceptual map and a keyword co-occurrence network, to identify the main research areas and groups involved in Cripto research. This pipeline is aimed at assisting in the creation of a research density map, integrating data from different fields and identifying converging research questions.

Chapter 6 concludes the work, providing a thematic analysis spanning the presented studies and the current scientific landscape to contextualize the reported findings. Particular attention is dedicated to addressing open questions and issues specific to bone metastatic prostate cancer and to precision medicine as a valuable clinical approach.

Collectively, this work demonstrated the feasibility and clinical applicability of organoids for the development of near-patient pharmacological assays in advanced prostate cancer. A research pipeline was presented, introducing and characterizing novel tools for the study of advanced prostate cancer and for the development of translational assays compatible with clinical decision making.