Tumor heterogeneity in glioblastoma

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Summary

Glioblastoma (GBM) is the most aggressive type of primary brain cancer in adults which poses multiple clinical challenges. At present, standard-of-care treatment consist of neurosurgical resection followed by an intensive schedule of chemotherapy and radiotherapy. This treatment schedule aims to prolong a patient's life as GBM is considered to be an incurable cancer, which inevitably recurs with limited treatment options available at recurrence. Importantly, the current standard-of-care has remained unchanged over the past 15 years, despite multiple clinical trial efforts, which emphasises the large clinical need for new treatment options.

Several factors can be attributed to the lack of success of clinical trials that have been conducted in GBM research. GBM is characterized by its diffuse growth pattern throughout the healthy brain parenchyma, which extends beyond the macroscopic tumor borders that can be visualized by current imaging modalities. This implies that a complete resection of the tumor can never be achieved and radiotherapy is limited by normal tissue toxicity. Also, the location of GBM inside the brain parenchyma provides several challenges. The blood-brain-barrier (BBB) is very efficient in isolating the brain from the systemic circulation, preventing novel treatment options such as monoclonal antibodies from adequate penetration into the tumor. In addition, the unique, brain specific immune micro-environment and microglia/macrophage oriented immunosuppressive environment in GBM can explain the lack of success of T-cell oriented immune checkpoint inhibitors which have seen great success in other solid cancers.
Additionally, the presence of tumor heterogeneity is widely accepted to be on the major determinants of treatment failure and poor prognosis seen in GBM patients.

Tumor heterogeneity implies there are major differences in tumor characteristics between patients (intertumoral heterogeneity) as well as the occurrence of multiple different subclones within one patient (intratumoral heterogeneity) each with different biological and molecular characteristics, ultimately leading to differences in tumor growth and treatment response. Tumor heterogeneity can also adapt over time due to changes on the genetic level, in example under the influence of factors in the tumor micro-environment such as hypoxia. Similarly, treatment-induced genetic alterations and changes of the tumor micro-environment can occur which changes GBM biology over time.

This thesis focusses on the tumor heterogeneity in GBM and explores novel methods to study tumor heterogeneity in GBM. In pre-clinical and translational cancer research, patient-derived cancer organoids have been developed as a model which maintains tumor heterogeneity. Therefore, patient-derived cancer organoids have an improved resemblance of the actual tumor when compared to traditional cell line models.
Patient-derived cancer organoids are stem-cell based three-dimensional cell culture models which retain self-renewal properties and are able to undergo multilineage differentiation, therefore maintaining intratumoral heterogeneity.

Chapter 2 describes the previous efforts that have been made on patient-derived cancer organoids throughout all different types of cancers. This chapter focuses on how these cancer organoids are being used to predict treatment response and how they can aid in selecting the most optimal treatment strategy. We describe 60 studies in which cancer organoids have been developed for a wide variety of solid cancers in which most studies use cancer organoids for drug screenings. In a few studies, the drug response of the organoids was directly compared to the actual clinical treatment response. These studies did show promising results on their ability to predict whether a patient will show a response to a specific treatment. However, these studies are still very limited and larger trials, as well as standardization of culture protocols and measurement of drug response is needed before such approaches can actually be moved towards clinical implementation. Importantly other

aspects which influence treatment response such as the tumor micro-environment should be accounted for as well in such models to improve its resemblance of the actual tumor and therefore its predictive value.

In Chapter 3 we set out to develop a patient-derived GBM organoid (PGO) model to study tumor heterogeneity in GBM. PGOs were developed by culturing GBM cells acquired from surgical resection in an extracellular matrix. We showed that these PGOs retain genetic mutations that were present in the original tumor and also maintained intratumoral heterogeneity on the single-cell level. PGOs were also suitable models to test the response towards chemotherapy (temozolomide) and radiotherapy. Interestingly, by comparing gene expression levels in PGOs before and after chemotherapy we were also able to identify the JNK pathway as a possible actionable target and determinant of sensitivity towards temozolomide. By treating PGOs with a combination of temozolomide and a small molecule JNK pathway inhibitor we showed an improved treatment response of the combination treatment providing additional rationale for future studies on JNK inhibition in the treatment of GBM.

Besides pre-clinical approaches to improve understanding about tumor heterogeneity in GBM, novel approaches on using clinical data are also being developed. A lot of attention has been given towards utilizing novel approaches in imaging modalities such as magnetic resonance imaging (MRI) which are routinely acquired in GBM patients. Besides qualitative imaging features, such as tumor size or contrast enhancement, MR images contain more information beyond what can be observed. Computational approaches, commonly called radiomics, have been developed to extract quantitative imaging features on textures, shapes and intensities. Data gathered from this type of analysis can be used in artificial intelligence and deep learning models to develop prognostic and predictive models when combined with clinical patient data.

Chapter 4 describes non-invasive diagnostic modalities that are currently available for GBM. Non-invasive diagnostic modalities such as nuclear imaging, MRI and liquid biopsies are possible strategies to acquire information on tumor characteristics. Detection levels of circulating tumor cells or other circulating biomarkers in both the blood and cerebrospinal fluid are low and the lack of validated biomarkers as well as the necessary expertise and equipment for such analysis are major obstacles for the clinical implementation of liquid biopsies. Specific positon-emission tomography (PET) tracers have been studied as biomarkers for specific molecular characteristics of the tumor but are also not widely available.

As MRI is the most widely used diagnostic modality this holds the greatest promise in non-invasive diagnostic methods. Associations between imaging features and patient prognosis as well as molecular markers have been made in multiple studies. However, the large heterogeneity between studies on this subject impedes a direct translation towards the clinic.

In Chapter 5 we set out to develop a prognostic and predictive model using integrated quantitative and qualitative imaging analysis in GBM. This study showed that combining clinical data with quantitative and qualitative MRI imaging features resulted in the most optimal prognostic model for overall survival which could be replicated in an external validation cohort. Using these features to predict molecular alterations present in the tumor was promising after development but this could not be reproduced in an external validation cohort in a clinically relevant manner.

The previously described efforts mainly focus on cancer cells and their role in tumor heterogeneity and treatment response. Whilst, cancer cells and their biological and molecular characteristics are of course a major determinant of treatment response and patient outcome, the tumor micro-environment has gained a lot of attention in cancer research. With the emergence of immune checkpoint inhibitors and its major success in solid cancers, such approaches have also been studies in GBM but have so far only given disappointing results in clinical trials. Chapter 6 describes the first

results of our multiplex single-cell immunohistochemistry analysis on a patient cohort of newly diagnosed GBM. This will provide the opportunity to study the interaction between cancer and immune cells and also thoroughly characterize the immune cells which are present in the tumor. This study will attempt to improve understanding of the immune micro-environment on the single-cell level and to identify potential strategies to improve success of immunotherapy in GBM.

In summary, this thesis describes approach to study tumor heterogeneity in GBM in both the pre-clinical and clinical setting. We showed that PGOs are promising novel GBM models that maintain intratumoral heterogeneity. The work in this thesis provides opportunities for future research on PGOs in GBM such as incorporating components of the tumor micro-environment. Also we show the possible application of computation approaches such as radiomics to develop prognostic and predictive models. Nonetheless, both techniques require standardization, optimization and further validation before they can be translated towards clinical implementation and actually influence GBM patient care.