English Summary

Pioneering Preclinical Research in Diffuse Intrinsic Pontine Glioma: Towards New Treatment Strategies

Circa 70-80% of brainstem gliomas originate in the pons, and are named diffuse intrinsic pontine gliomas (DIPG). With a median overall survival of less than one year, DIPG is the pediatric malignancy with the worst prognosis, basically leaving no chance of survival. While in the past thirty years considerable improvement in survival of pediatric patients with cancer has been achieved, no progress has been made for children affected by DIPG. Tumor resection is not an option for these patients, given the highly diffuse nature of this tumor and the delicate anatomical area where it is located. In addition, since the advent of high quality MR imaging, the necessity for biopsy became questionable to achieve a diagnosis and, therefore, no primary DIPG material was available in the laboratory for research. As a consequence little is known on the biology of this tumor and very few preclinical models are available for translational research. Hence the design of DIPG clinical trials has been mainly based on results obtained in clinical studies for adult glioma patients or for pediatric patients bearing other types of brain tumors. To date, no therapeutic agent has shown survival benefits and radiotherapy (RT) remains the only, albeit transient, effective treatment for this patients.

The thesis presented here discusses the pioneering work done to setup preclinical DIPG models (scope 1), as well as to identify new treatment strategies for DIPG that could be readily translated in the clinic (scope 2).

In chapter 1, we introduce the thesis, describing literature related to the present work and we discuss the motivations and aims of the studies performed.

In chapter 2, we present the development and characterization of a DIPG mouse model. When we started with the work presented in this thesis, no primary DIPG cell culture was available. Therefore, in order to develop a DIPG mouse model, we employed the E98 adult glioma cell line, given its highly infiltrative growth pattern in vivo. Human E98 glioma cells were stereotactically injected into the pons of nude mice. The E98 DIPG tumors presented a strikingly similar histopathology compared to autopsy material of a DIPG patient, including diffuse and perivascular growth, brainstem- and supratentorial invasiveness and leptomeningeal growth. Next, the E98 cells were cultured in vitro and engineered to express firefly luciferase and mCherry. Interestingly, after injection of these cultured E98-Fluc-mCherry (FM) cells into the pons of nude mice, focal instead of diffuse pontine glioma developed. However, the E98 DIPG infiltrative phenotype was restored when cells were injected into the pons directly after an intermediate subcutaneous passage. The diffuse E98-FM model was subsequently used to test escalating doses of irradiation, applying the bioluminescent Fluc signal to monitor tumor recurrence over time. Altogether, this chapter describes an accurate DIPG mouse model that can be of clinical relevance for testing experimental therapeutics in vivo.
In chapter 3 we discuss the implementation of a nation-wide autopsy protocol in the Netherlands to collect primary DIPG material. To date six autopsies have been performed. We succeeded to obtain DIPG tumor tissue with a short post mortem delay and were able to develop one of the first DIPG cell cultures reported in literature. Importantly, we show that none of the parents regretted their choice to participate, and they all derived comfort in donating tissue of their child in the hope to help future DIPG patients. Additionally, we describe in detail the methods of the entire procedure starting from guidelines for attending physicians, logistics for the day of autopsy, the autopsy procedure in itself, processing of primary tissue and the parents evaluation form. In conclusion, we demonstrate that obtaining post mortem DIPG tumor tissue for research purposes is feasible with short delay, and that the autopsy procedure is satisfying for participating parents and can be suitable for the development of preclinical DIPG models.

In chapter 4, we discuss inhibition of WEE1 kinase as a potential strategy to enhance DIPG response to RT. WEE1 kinase controls the G2 cell cycle checkpoint allowing for repair of irradiation (IR)-induced DNA damage. Here we demonstrate that WEE1 is overexpressed in DIPG tissue and cell cultures. We employed the clinically relevant WEE1 inhibitor, MK-1775, to investigate its radiosensitizing effects in in vitro and in vivo DIPG models. Using the orthotopic E98-FM DIPG mouse model, we demonstrate that MK-1775 has radiosensitizing activity in vivo. Altogether, these results show that inhibition of WEE1 kinase in conjunction with RT holds potential as a therapeutic approach for the treatment of DIPG.

In chapter 5, we report our first experiments with convection-enhanced delivery (CED) of adenoviruses in the rat supratentorial brain. CED is an interstitial local delivery technique that uses hydrostatic constant pressure to propel therapeutic agents over relevant anatomical volumes. This study was carried out in preparation of a clinical trial now ongoing in the Netherlands and employing oncolytic adenoviruses to treat adult glioma patients. In this chapter we show that CED of adenoviruses in the rat brain is feasible when regional anatomical differences are taken into account. In fact, while the volume of distribution is related to infused dose in the gray matter of the corpus striatum, it appears to be related to infusion volume in the white matter of the corpus callosum and external capsule. Finally, we demonstrate that super paramagnetic iron oxide nanoparticles infusion could be considered to validate proper catheter positioning and predict adenoviral distribution in the brain.

Further, we used the knowledge gained in chapter 5, to set up CED in the mouse brainstem, a study described in chapter 6. Here we show that carmustine, our CED candidate, was highly effective on primary DIPG cultures. In addition, CED of carmustine into the murine brainstem was non-toxic and significantly increased survival in mice bearing two orthotopic DIPG models, i.e. 1) the newly developed mouse VUMC-DIPG-3 model comprised of spontaneously transformed Nestin+/GFAP-/Olig2+/SOX2+ host cells after injection of human DIPG cells, and 2) the previously established human E98-FM DIPG model.

In chapter 7 we discuss the work and results presented in this thesis. In addition, we highlight interesting research avenues for DIPG research.