Chapter 1

General Introduction
Gliomas comprise the majority of intrinsic cerebral tumors and arise from neuroglial cells. The most common gliomas arise from astrocytes (or precursors) and are called astrocytomas. The World Health Organization has graded them from grade I (benign) to grade IV (malignant). The grade IV astrocytoma is frequently called glioblastoma (GBM). GBM has a dismal prognosis and after current standard therapeutic regimen of (1) surgery, (2) radiotherapy and (3) chemotherapy with temozolomide, the overall survival reaches a median of only 14.6 months. Although conventional therapies such as maximal safe surgery, radiotherapy, chemotherapy and combinations thereof contributed to improvement of the overall survival, these treatment modalities seem to have reached a survival plateau. Therefore, there is an urgent need to explore new approaches that can lengthen overall survival of GBM patients.

Nowadays many new anti-cancer approaches are based on extensive knowledge of cellular pathways and new anti-neoplastic agents are developed to interfere within these intracellular pathways on a “targeted” basis. Examples of such targeting strategies are: -1) blocking the EGF receptor (EGFR) with anti-EGFR monoclonal antibodies (Cetuximab) thereby inhibiting EGFR signaling; - 2) disrupting the receptor catalytic activity and inhibiting EGFR autophosphorylation and downstream signaling by targeting the intracellular catalytic domain of EGFR tyrosine by tyrosine kinase inhibitors (erlotinib and gefitinib); - 3) the use of antisense molecules inhibiting translation of the EGF; - 4) the use of small molecule inhibitors against anti-apoptosis proteins, such as Bcl-2 and Bcl-X_L; - 5) inhibiting transcription of several anti-apoptotic proteins (IAP’s) by histone deacetylase inhibitors in order to bypass resistance to apoptotic signaling; - 6) inducing apoptosis by Tumor Necrosis Factor (TNF) or its related cytokines.

In this thesis we focus on a TNF related apoptosis inducing ligand (TRAIL) which can be targeted to its cognate death receptors, thereby activating the so-called extrinsic apoptotic pathway. In general, apoptosis is a biological regulative process for an organism to dispose needless or dangerous cells, without causing damage to the surrounding environment. This distinctly differs from necrosis, which is an uncontrolled process that leads to inflammation and in the brain to cerebral edema.

The death inducing ligand, TRAIL can be considered a new non-conventional anticancer drug, which has the potential to induce apoptosis in glioma cells without being neurotoxic. TRAIL (also called Apo2L) is a member of the tumor necrosis factor (TNF) cytokine family which includes FasL (CD95 L) and TNF-α. TRAIL induces apoptosis after crosslinking the death inducing receptors TRAIL-R1 or TRAIL-R2 and thereby activating the extrinsic pathway. In contrast, chemotherapy and radiotherapy activate the intrinsic pathway through DNA damaging.

TRAIL is present as an endogenous cytokine in the human body but its physiological role is not yet fully understood. Functionally, TRAIL has shown to selectively kill tumor cells, while normal cells are left unharmed, this in contrast to TNF-α which is
highly hepatotoxic. In vitro, and animal in vivo studies have shown potent apoptosis inducing activity towards various tumor cell lines including GBM cell lines and as such TRAIL has gained substantial research interest during the last decade. In this thesis several aspects of TRAIL receptor-mediated apoptosis induction in GBM are described and possible opportunities to exploit TRAIL-derivatives against primary brain tumors are discussed.

Aims and outline of the thesis

In order to determine if TRAIL is applicable as an anti-neoplastic agent in patients with a GBM knowledge on the presence of TRAIL receptors in malignant astrocytic tumors or normal tissues is important. Also understanding how and to what extent TRAIL induces apoptosis in GBM is essential. Therefore, the mechanism of TRAIL induced apoptosis must be understood. Understanding of the apoptosis pathways in glioma cells is important to predict sensitivity of these tumors for TRAIL. Resistance of GBM to TRAIL can impede the therapeutic efficacy of TRAIL. This resistance may be the result of defects in the apoptosis signaling pathways. Detailed knowledge on defects in the apoptosis pathways in GBM cells may be useful to predict sensitivity for TRAIL-based approaches.

Chapter 2 represents a review on TRAIL biology and elaborates on the presence of TRAIL receptors in normal tissues and gliomas. Beneficial effects of combinational treatment of TRAIL with conventional therapies are discussed. Resistance pathways of tumor cells for TRAIL and possible solutions to overcome TRAIL resistance are discussed. Moreover, novel approaches to selectively target soluble TRAIL to pre-selected tumor-associated antigens of cancer cells are reviewed.

Data on the presence of death inducing TRAIL receptors on primary GBM cells is sparse\(^2,3\). Research done on glioma cell lines, which differ for primary tumor tissue, show the expression of both death inducing receptors TRAIL-R1 and TRAIL-R2\(^4\). However if TRAIL is to be used as anticancer drug for GBM it is important that TRAIL receptors are present on primary GBM cells. Therefore the question arises: are TRAIL receptors present on primary GBM tissue and does the amount of expression correlates with survival? In Chapter 3 the semi-quantitative and quantitative expression of TRAIL receptors on primary GBM tissue and its association with patient survival are described.

In Chapter 4 the novel development of a TRAIL fusion protein (scFv54:sTRAIL) with specificity for the epidermal glycoprotein 2 antigen is described. “Targeting” drugs to the site of the tumor is a principle by which a drug is specifically directed to tumor associated antigens, present on the cell membrane of the tumor cells. The rationale
is to deliver an optimal dose of drugs at the site of the tumor. Furthermore, in vitro studies have shown that TRAIL-R1 has distinct other properties than TRAIL-R2. It has been shown that TRAIL-R2 can only be activated by multiple TRAIL molecules which are interconnected with each other (crosslinked) by a crosslinking enhancing tag. In contrast TRAIL-R1 can be activated by both non-crosslinked and crosslinked TRAIL. However it has been shown that tagged-TRAIL can alter the structure of the receptor resulting in hepatotoxicity. Therefore TRAIL alternatives have been developed with the capacity to be specifically targeted to the tumor cell and also having the capacity to crosslinking TRAIL receptors thereby activating both the TRAIL-R1 and TRAIL-R2 receptor. Questions addressed were; is it possible to engineer a TRAIL fusion protein with targeting activity toward a tumor associated antigen and does this specific targeting lead to an enhanced death inducing capacity? Also the possible advantages of tumor-selective targeting of TRAIL are discussed in chapter 4.

Systemic application of TRAIL has the disadvantage that TRAIL must cross the blood brain barrier in order to eliminate tumor cells. It is questionable whether systemic administration of TRAIL will lead to a relevant concentration in the tumor and the peritumoral region. A possible method to bypass this problem is delivering TRAIL intracerebrally, preferentially within the tumor or the peritumoral area. A technique for delivering TRAIL in the vicinity of the tumor is through intracerebral implantation of encapsulated TRAIL producing cells. Studies concerning the application of alginate encapsulated producer cells to cure neurodegenerative diseases and brain tumors have been published with various success rates. Insufficient biocompatibility of the capsules and subsequent death of the encapsulated producer cells has hampered the success and clinical application of this technology. Recent advances such as application of pure alginites with a defined composition has brought new insight in the factors determining the biocompatibility of the capsules. In Chapter 5 a newly developed fusion protein “scFv425-sTRAIL” was used. The manufacturing of scFv425-sTRAIL producing cells and their microencapsulation in pure alginites was evaluated. Also the biological properties of the TRAIL producing cells after encapsulation were assessed. A mouse brain model was used to evaluate the biocompatibility of the alginate capsules after intracerebral implantation. Furthermore the efficacy of alginate encapsulated scFv425-sTRAIL producing cells and the potential of this method for targeted delivery of a TRAIL fusion protein to brain tumor cells is discussed in chapter 5.

Another method to deliver drugs to the site of the tumor is the convection enhanced delivery (CED) technique. CED of drugs to the site of the tumor is an extensively studied method. It has been proven effective in several in vitro and animal in vivo studies. Several Phase III studies addressing the issue to target toxins with CED to GBM were initiated (multicenter trial Phase III, PRECISE study; CED of IL13-Pseudomonas exotoxine intracerebrally and phase III study TransMID; Tf-CMR107; Transferrin-
Diptheria tox). Based on the results in the literature it seems logical to explore the possibilities of convection enhanced delivery of a TRAIL fusion protein targeted against the EGF receptor (scFv425:sTRAIL) in a murine brain tumor. Therefore we needed to address the following issues; can we identify a cell line which is extremely sensitive to the scFv425:sTRAIL fusion protein? Can this cell line be implanted in a mouse brain resulting in a high probability of acceptance? If we use a CED technique for delivering the TRAIL fusion protein does it show any efficacy in a certain mouse brain tumor model? In Chapter 6 these questions are considered and partially answered.

Currently, the standard treatment for patients with a GBM is surgery followed by radiotherapy and chemotherapy. Although this therapy scheme has lengthened survival, still there are glioma cells which show resistance or acquire resistance to either therapy. Also resistance of glioma cells to TRAIL is a know feature. It has been suggested, in non-GBM cell lines, that radiation can enhance the apoptosis-inducing efficacy of TRAIL\textsuperscript{11-16}. Whether or not this is a general effect seen in all TRAIL receptor-positive cell lines and whether or not this also enhances the ultimate loss of clonogenicity of tumor cells remains to be elucidated. It would be interesting to evaluate if TRAIL in combination with radiotherapy would lead to enhanced apoptosis in cancer cells thereby bypassing resistance and leading to prolonged survival. In Chapter 7 we evaluated the effect of combined α-radiation-TRAIL therapy in a glioblastoma cell line, measuring both early apoptotic cell death and clonogenic ability as endpoints.

A general discussion is given in Chapter 8, followed by some thoughts on future perspectives. Chapter 9 summarizes the thesis.
References
