ABSTRACT

Glioblastoma multiforme (GBM) is a lethal malignant primary brain tumor in adulthood, characterized by several genetic alterations and cellular heterogeneity (Louis DN, 2016).
The standard treatment consists of a combination of surgery, radiotherapy and chemotherapy. Despite the aggressive treatments, a definitive therapy is not available at present, and the mean patient survival time reaches only 14.6 months (Wilson TA, 2014).
Temozolomide (TMZ), is an alkylating agent currently used as first line therapy in combination with radiotherapy (Zhang J, 2012). Considering drug-resistance issues, as of now, TMZ used alone or in combination with radiotherapy can only increase the lifetime expectancy of GBM patients. The enhanced activity of a DNA repair enzyme, the O(6)-methylguanine-DNA-methyltransferase (MGMT), represents one of the main mechanisms of drug resistance in glioma, since it repairs TMZ-induced DNA lesions (Fan CH, 2013).
Gliomagenesis is attributed to many molecular changes involving both genetic and epigenetic mechanisms; thus, modifications in the apoptotic pathways may not only contribute to the develop of the tumor, but also to the resistance towards classical genotoxic approaches of therapy (Adamson C, 2009).
Further, microRNAs, a class of small non-coding RNAs, play a pivotal role in the development of a malignant phenotype of glioma cells, cell survival, proliferation, tumor angiogenesis and metastasis. Tumorigenesis occurs as the result of imbalances between oncomiRNAs and tumor-suppressor miRNAs, both acting as gene regulators at post-trascriptional level by either repressing translation or degradating the target mRNA. For instance, the oncomiRNAs microRNA-155 and microRNA-221, are significantly elevated in GBM, downregulating multiple genes associated with cancer cell proliferation, apoptosis, invasiveness and drug resistance (Liu Q, 2015; Xie Q, 2014; Zhang CZ, 2010; Shea A, 2016).
Therefore, new therapeutic targets and tools should be developed based on a better understanding of the molecular pathogenesis of glioma. In particular, it would be interesting to design new therapeutic approaches that enhance currently available treatments and/or limit tumor growth, as well as reducing resistance to chemoterapeutic drugs.
The aim of this PhD thesis was to develop novel possible therapeutic interventions

inhibiting the growth of the tumor cells, as well as inducing apoptotic cell death by sensitizing glioblastoma cells to temozolomide treatment and/or potentiating its activity. In light of this aim, human U251 and TMZ-resistant T98G glioma cells were studied combining: (i) the treatment with temozolomide and corilagin, an interesting tannin extracted from plants of the *Phillantus* family, and (ii) temozolomide with anti-miR-221 and anti-miR-155 PNAs. Corilagin (COR) is known to exhibit antioxidant, anti-inflammatory and antitumor activity. It was reported to induce biological effects by interfering with the anti-apoptotic NF-kB transcription factor (Gambari R, 2012; Dong XR, 2010) and to induce cancer cell apoptosis (Jia L, 2013, Ming Y, 2013; Gu Y, 2016). Therefore, corilagin could potentially work as an active compound for treating glioblastoma. Firstly, by docking studies, it was demonstrated that corilagin (COR) is able to suppress the level of NF-kB by preventing its ability to bind with DNA. Upon COR treatment, U251 and T98G glioma cells showed a reduction in their proliferation, but most importantly showed increased apoptosis. Interestingly, when using T98G glioma cells, these showed resistance to TMZ treatment, and when co-treated with TMZ plus COR, they reached a level of apoptosis higher than that with temozolomide or corilagin alone. This is probably related to the fact that corilagin, but mainly corilagin with temozolomide (COR + TMZ), decrease the expression of MGMT mRNA and induced caspase-3 (CASP-3) activation; moreover, corilagin demonstrated to be active at inhibiting cell migration, particularly when combined with temozolomide.

As regards to targeting microRNAs to modulate cancer behaviour, peptide nucleic acids (PNAs) against miR-155 and miR-221 were also considered by this thesis. PNAs are synthetic nucleic acid analogues wherein the negatively charged sugar-phosphate backbone is replaced with charge-neutral amide linkages; they are considered ideal candidates for application as antisense therapeutics that block expression of complementary mRNA (Nielsen PE, 1991; Larsen HJ, 1999). MiR-155 and miR-221 were selected from a long list of microRNAs dysregulated in GBM, since they were predicted to target caspase-3 mRNA, an important factor involved in the apoptosis pathway. The results obtained from the Bio-Plex analysis and from the Caspase 3/7 assay, after treatment of glioma cells with PNAs R8-PNA-a155 and R8-PNA-a221, confirmed our hypothesis that CASP-3 is a target gene of miR-155 and miR-221. More recently, within our group, it was demonstrated that R8-PNA-a221 has pro-apoptotic effects in glioma cells (Brognara E, 2016); thus, also the effect of PNAs R8-PNA-a155 was investigated on both U251 and T98G cells. The

obtained data showed that both PNAs are selective in targeting their relative microRNA and they are able to induce apoptosis in both the glioma cell line models under study.

Since oncomiRNAs promote cancer cell growth and survival, we investigated whether, in TMZ-resistant T98G glioblastoma cells, combining temozolomide with PNAs could have a greater effect than single treatments. Co-administration of R8-PNA-a221 or R8-PNA-a155 induced apoptosis of TMZ-treated T98G cells at a level higher than that obtained following singular administration of R8-PNA-a221 or R8-PNA-a155; similar was the effect observed on apoptosis involving caspase-3 activation.

In conclusion, the results reported in this PhD thesis demonstrate that apoptosis induction, could be an efficient way to modulate the progression of cancer and the drug resistance. With this research, we showed that using natural compounds or synthetic agents, such as corilagin and PNAs, respectively, could constitute an efficient and powerful strategy to render resistant-glioma cells more susceptible towards therapy-induced apoptosis, as well as improving the efficacy of the first-line drug temozolomide treatment.