Proton therapy for glioblastoma multiforme: preclinical radiobiological studies and evaluation of gold nanoparticles as radiosensitizers

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Introduction:

Glioblastoma multiforme (GBM) is the most prevailing primary brain malignancy in adults and presently remains a fatal condition[1]. Hence, proton therapy has been presented as an alternative to conventional radiotherapy treatment in GBM, since its dose-depth curve offers efficient tumor damage while avoiding undesired radiation exposure to normal brain tissue. Adding an extra FLASH effect to proton irradiation would also reduce neurotoxicity as opposed to standard low-dose exposure[2]. Complementarily, the use of gold nanoparticles (AuNPs) to improve radiotherapy effectiveness has also been proposed for glioblastoma tumors, since AuNPs act as local radiosensitizers upon interaction with external radiation due to the release of Auger electrons[3]. The first goal of this work is to optimize a PET cyclotron-based FLASH proton beam for *in vitro* research due to the lack of biological data in this area. Later on, glioblastoma cells will be exposed to Co-60 γ-radiation, kilovoltage X-rays and to the proton beam for evaluation of radiobiological effects in the presence and absence of the AuNPs.

Methods

A PET cyclotron-based FLASH proton irradiation set-up was assembled at ICNAS -University of Coimbra (figure 1)[4]. The dosimetry of the system was assessed using a calibration curve from a standard radiotherapy LINAC applied to proton-irradiated EBT3 films, allowing to establish a direct relationship between the measured integrated beam charge and the absorbed dose at the target. FLASH dose rate measurements were performed by determination of target exposure time. Later on, the glioblastoma cell lines U87 and U373 were exposed to 160 kVp X-rays, Co-60 γ-radiation and protons to compare effects on cell survival. Finally, U373 cells were subjected to a combined treatment including different types of radiation and AuNP incubation to quantify dose enhancement effects. Two types of AuNPs were evaluated in this study: GBM targeted AuNP-BBN (bombesin) and their untargeted counterparts AuNP-DOTA[5].

Results

A calibration for the FLASH proton irradiation system was successfully determined by associating the proton dose at the target to the integrated beam charge. FLASH measurements yielded a dose rate of 13.5 Gy/s. Irradiation experiments without AuNPs showed that U373 cells are less radiosensitive to γ-radiation than to X-rays and FLASH protons. An identical outcome was observed for the U87 cell line for doses of photon radiation beyond 2 Gy. AuNP-BBN produced a dose enhancement effect in U373 cells when combined with kilovoltage X-rays and FLASH protons as opposed to Co-60 γ-radiation. Additionally, AuNP-DOTA did not enhance radiation effects on U373 cells, confirming that the presence of the bombesin peptide is crucial to promote specific uptake and local radiosensitization.

Conclusion

A PET cyclotron-based FLASH proton beam line was successfully optimized for radiobiological *in vitro* research. We also presented concomitant GBM-targeted AuNP-BBN and radiotherapy as a novel treatment modality for glioblastoma, as AuNP-BBN induced selective and local radiosensitization. Future work will include additional proton beam characterization with Monte Carlo simulations, as well as further proton irradiation studies with other cancer cell lines and complementary AuNP radiosensitizing studies.

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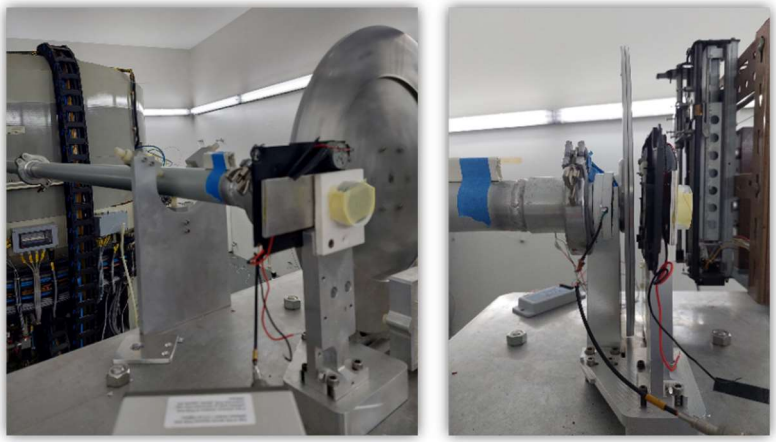

a) b)

Figure 1: Overall view of the PET cyclotron-based FLASH proton beam line with application in radiobiological *in vitro* research including a cell container holder (a) as well as a transversal perspective of the target site and the electronics for beam current assessment (b).