Carborane based Carbonic Anhidrase IX inhibitors: a potential target for BNCT of malignant pleural mesotheliomas and breast cancer.

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The large majority of chemotherapeutic protocols and radiotherapies can considerably reduce tumour masses, but they often fail in causing their complete regression as shown by a high number of tumour recurrence cases. Moreover, the time-dependent development of chemoresistance and radioresistance by a minor cell population within the tumour and the nonspecific toxicity toward normal cells are the other major limitations of standard therapies. For these reason, in recent years, much attention has been devoted to the use of combinations of different therapeutic modalities to treat cancer. Exploiting this approach, BNCT has been proposed as therapy strategy combined with the inhibition of carbonic anhidrase IX (CAIX) enzyme.
Carbonic anhydrase IX (CAIX) is a hypoxia-inducible enzyme that is overexpressed by cancer cells and plays an important role in tumour acid-base homeostasis by promoting cancer cell survival in hypoxic microenvironment. Recently, a relationship between CA overexpression and tumour cells resistance to chemio or radio therapy has been evidenced and novel antitumour therapies based on the use of CAIX inhibitors are under study in clinical. The ambition of this study is to establish a synergic therapeutic approach, arising from the combination of a preliminary enzymatic inhibition followed by neutron irradiation exploiting a sulfonamide functionalized carborane (CA-SF) [1] with respect to BNCT used alone in *in vitro* studies on breast cancer (MCF7) and malignant mesothelioma (AB22, AE17, ZL34) cells. In order to assess whether the combination of BNCT and CAIX enzyme-inhibitory capacity resulted in an improvement of the treatment outcome with respect to BNCT given as monotherapy, the clinically used boron delivery agent sodium BSH was exploited as an alternative boron source. Firstly, the cells were incubated with increasing concentration of CA-SF or BSH to assess whether the amount of boron internalized by cells was enough to allow BNCT and in the same time the cytotoxic effect of CA-SF or BSH was evaluated on cells. ICP-MS analysis showed that both mesothelioma and breast cancer cells internalized enough CA-SF to perform BNCT (50 µg/gr boron).Furthermore, increasing the concentration of CA-SF, the percentage of viable cells proportionally decreased demonstrating its toxic effect on cells overexpressing CAIX enzyme. The internalization of BSH, as expected, was significantly less efficient with respect to CA-SF, and 50 µg/gr of B were reached only upon incubation in a concentration range 1-5 mM, that was more than one order of magnitude higher with respect to the same range used for CA-SF incubation and it didn’t affect the cell viability that was higher than 80%. The effect of BNCT after BSH or CA-SF treatment was evaluated by the proliferation rate of cells surviving to irradiation. Only survived cells treated with the CA-SF before neutron irradiation showed a marked inhibition of proliferation. This result demonstrate the important role of the CAIX

inhibitor in the proliferation of these tumour cells after BNCT treatment. The enzymatic and BNCT treatment of a subcutaneous tumour mouse model of malignant mesothelioma is under study.

References

1. J. Brynda et al. Carborane-based carbonic anhydrase inhibitors. Angew Chem Int Ed Engl. 2013;52(51):13760-3