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Euro Cancer 2019: Analysis of DNA damage responses and repair mechanisms after boric acid-mediated boron neutron capture therapy in hepatocellular carcinoma- Kuan-Hao Chen- National Tsing Hua University

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Short Communication

Background: Boron neutron capture therapy (BNCT) is a two-step radiation treatment modality, which kills tumor cells and leaves normal cells undamaged. In previous studies, boric acid (BA)-mediated BNCT has demonstrated its therapeutic efficacy in treating hepatocellular carcinoma (HCC) in rat and rabbit models. However, the DNA damage responses and repair mechanisms induced by BA-BNCT in HCC remain unclear.

Aim: This study thus aims to investigate whether the BA-BNCT induced DNA double-strand break (DSB) and to explore which DSB repair pathways, homologous recombination (HR) and non-homologous end joining (NHEJ), would be the primary pathway.

Methods: Huh7 (human HCC cell line) was pre-treated with BA 30 minutes before exposing to neutron irradiation at Tsing Hua open pool reactor in National Tsing Hua University, Taiwan. Afterwards, cells were harvested for immunocytochemistry and immunoblotting analysis. Hepatocellular carcinoma (HCC) is one of the most common types of cancer in Southeast Asia, Africa, and Southern Europe. It is also the second most common cancer in males and females in Taiwan. Surgery is the best treatment for patients with a focal liver tumor. However, HCC is usually diagnosed late in the course of the disease, and at the time of diagnosis, more than 80% of patients have vascular invasion and multifocal tumor. The multi-port irradiation that is usually utilized in HCC treatment delivers a radiation dose that exceeds that which can be tolerated by normal liver, and may cause fatal liver failure. The most important issues in this field are the improvement of therapeutic efficacy and reduction of complications. An effective treatment method should be non-invasive and have few side-effects; it should also have minimal effects on normal liver tissue and allow for the rapid recovery of patients. Boron neutron capture therapy (BNCT) may be effective in reaching these goals.

It involves treating tumors with high linear-energy-transfer (LET) alpha and 7Li particles, resulting in significant damage to tumor cells from the nuclear reaction of 10B (n, α) 7Li. High LET means that irradiation has a high energy density, which breaks the double strands of DNA, and the use of short-range radiation ensures that adjacent normal tissues are spared from radiation-induced damage. However, BNCT does not satisfy the aforesaid requirements because boron drugs for use in the treatment of HCC are lacking. Certain criteria need to be considered for the BNCT treatment of HCC, including the low-toxicity of the boron drug, its high retention in the HCC tumor and its vessels but low retention in the liver and adjacent normal tissues, and sufficient thermal neutron fluency. Consequently, searching for an appropriate boron drug is the main aim in research on BNCT treatment for HCC at the Tsing Hua Open-pool Reactor, which can provide sufficient thermal neutron fluency. Borocaptate sodium (BSH) and boronophenylalanine (BPA) are the two boron drugs that are currently used for clinical BNCT. BSH is a water-soluble boron drug that has been used in clinical trials of BNCT for patients with glioma. However, the disadvantage of BSH-mediated BNCT for treating HCC is low tumor to liver ratio of boron concentration. The disadvantage of BPA-mediated BNCT in treating HCC is that adjacent tissue accumulates five to ten times more boron than the tumor. According to animal studies in which BPA was used as the boron drug, the pancreas, which is adjacent to the liver, accumulates a higher concentration of boron than does the liver tumor. Therefore, under neutron irradiation, when the liver tumor acquires a dose sufficient for BNCT treatment, the pancreas is severely damaged. Consequently, when BPA was used as the boron drug in a clinical trial of BNCT for the treatment of a liver tumor, the patient's liver had to be removed by surgery following drug injection to enable ex vivo neutron irradiation of the liver tumor. The liver was then autotransplanted back to the patient following neutron irradiation. This novel liver tumor therapy resulted in tumor complete response. However, such ex vivo neutron irradiation has certain limitations and risks. For example, its success depends on the technique used by the surgeon to remove the organ, the physical condition of the patient, and the efficacy of sterilization throughout the surgery. In vivo BNCT for liver tumor, which does not require for removal of the liver from the body, may be preferable. The first attempt to use BNCT in vivo to treat HCC has been made. Multiple tumors in the right liver lobe were treated with BNCT, which involved the intra-arterial administration of BPA and BSH through a catheter located in the right hepatic artery, followed by the injection of a mixed BSH/lipiodol emulsion. Tumors in the left lobe were treated with hepatic arterial chemoembolization. This method has significantly lower risk than that of ex vivo BNCT treatment for HCC. However, BNCT-treated tumors tend to recur 3.5 months after BNCT and the patient died from liver dysfunction 10 months after BNCT (15). Hence, new boron drugs for BNCT for the treatment of HCC need be developed.

Results: The expression of ?H2AX, a marker of DSB damages, was observed to peak at 4 h and diminished by 24h after BA-BNCT. The protein expression of BRCA1 and Rad51, both involving the HR pathway, were activated at 4 h. Surprisingly, BRCA1 sustained its activation to 48 h, while NHEJ-related proteins Ku70/Ku80 did not show significant changes after BA-BNCT.

Conclusion: These results suggested that DSB damages induced by BA-BNCT were primarily repaired through the HR pathway in HCC. Our findings could enable the identification of radio-sensitizer or adjuvant treatment by targeting the HR pathway, which could help to address treatment resistance and potentiate the efficacy of BA-BNCT for HCC.