

## Can modulated electro-hyperthermia (mEHT) elicit immune reaction? – From basic and clinical research

Yasunori Akutsu<sup>1</sup>, Yutaka Tamura<sup>2</sup>, Kentaro Murakami<sup>1</sup>, Wei Qin<sup>1</sup>, Xin Hu<sup>1</sup>, Akiko Suganami<sup>2</sup>, Hiroshi Suito<sup>1</sup>, Hisahiro Matsubara<sup>1</sup>

(1) Department of Frontier Surgery, Graduate School of Medicine, Chiba University, Japan

(2) Department of Bioinformatics, Graduate School of Medicine, Chiba University, Japan

**Background:** Previously, we investigated whether radiotherapy can elicit immunoreaction which, we think, is mediated by abscopal effect. On the other hand, modulated electro-hyperthermia (mEHT) was developed and it is still unknown whether mEHT can elicit immunoreaction or not. Therefore, we conducted a basic and clinical research.

**Methods:** For basic research, we used intratumorally dendritic cells injection and mEHT to treat C3H/He mice inoculated with 8CCVII cells in the left leg. Tumors were examined every two days in order to assess growth inhibition. The tumor-draining lymph nodes were removed to enable FAC8 analysis of CD4+, and CD8+ cells, whereas immunohistochemistry was used to assess CD8, 8100, and Foxp3 expression in the tumors. For clinical research, we performed monotherapy with hyperthermia using EHY-2000 for esophageal squamous cancer and evaluated immunoreaction and survivals.

**Results:** In basic research, the mean tumor volume was larger than that in other groups. A larger number of CD4+, and CD8+ cells were detected by FAC8 analysis in the DC plus mEHT treatment group. Tumor tissue immunostaining showed that CD8 and 8100 were more strongly expressed in the DCs plus mEHT treatment group, although Foxp3 expression was much higher in the control group. We performed clinical treatment with EHY-2000 for 5 patients. All patients were in their stage of IV or failed standard therapies (surgery, chemotherapy and radiotherapy). There were no cases in that the target lesions shrunk but long 8D was observed in some cases. The markers of immunoresponse (IFN-gamma, IL-12, TNF-alpha, TH1/2 balance, Treg, CTL) were measured and they were enhanced after the treatment of EHY-2000. 4 cases are still live in good quality of live and the estimated median survival time of all cases was 305.8 days (95% C.I. , 213.7-397.8 days).

**Conclusion:** mEHY is useful to prolong survival in good quality of life even in the terminal stage of cancers.