

## Study of Anti-proliferative Effects of Chaga Mushroom and Panax Notoginseng-containing Traditional Chinese Medical Supplement, Shibe-ria on Dog Bladder Cancer Stem Cells

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### 【Background】

Since the malignancy of dog bladder cancer (BC) is higher than human BC, most BC bearing dogs die early. In our laboratory, we established the method of bladder cancer organoid culture using the cancer stem cells in their urine samples. On the one hand, the usage of traditional Chinese medicine has been increasing in veterinary medicine. In our laboratory, we treated dog BC organoids with 39 kinds of traditional Chinese medicines. Among them, we clarified anti-proliferative effects of Chaga mushroom and Panax notoginseng-containing traditional Chinese medical supplement, Shibe-ria on dog BC stem cells.

### 【Object】

This study aimed to clarify anti-proliferative mechanisms and components of Shibe-ria on dog BC organoids.

### 【Methods and Results】

Dog BC organoids were treated with Shibe-ria (100  $\mu$ g/ml) for 6-24 hours and the expression of cancer stem cell markers was evaluated using Real time PCR. The expression of CD44 and SOX2 mRNA was significantly reduced. On the one hand, Dog BC organoids were treated with Shibe-ria extracted by various solvents (50-100  $\mu$ g/ml) for 72 hours, respectively. The cell viability of the organoids was assessed by Alamarblue assay. Shibe-ria extracted by 20 %, 50 %, 70 % methanol suppressed cell viability of dog BC organoids.

### 【Discussion】

We for the first time demonstrated that Shibe-ria inhibited the proliferation of dog BC organoids by suppressing expression of CD44 and SOX2 mRNA. Components in Shibe-ria extracted by 20 %, 50 %, 70 % methanol were suggested to inhibit the proliferation of dog BC organoids.