Hyaluronan synthase inhibitor induces apoptosis in canine mammary tumor cells through inhibition of spheroid formation

Jun Yamazaki, Shuta Ijichi, Runa Yoshida, Yuri Kimoto, Yuta Inoue, Moe Watanabe, Marina Yamada, Teruyoshi Saito

Lab. Vet. Pharmacol., Dept. Vet. Med., Nihon Univ. Coll. Biores. Sci.

Hyaluronan (HA) is one of the main components of the extracellular matrix. HA synthase (HAS) and hyaluronidase (HYAL) isoforms have been shown to influence malignant potential in cancer cells. Formation of a cohesive multicellular group has been reported to facilitate cancer progression, leading to distant metastasis without apoptosis. Previously, we demonstrated that the HAS inhibitor 4-methylumbelliferone (4-MU) inhibits cell proliferation and mobility. In this study, we used the canine mammary tumor cell line AZACB to investigate whether inhibiting HA production reduces cancer spheroid formation and induces apoptosis. In AZACB cells cultured under standard conditions, 4-MU decreased HA production, cell proliferation, and mobility, and increased Bim expression as an apoptosis marker. In addition, 4-MU inhibited the expression of HAS2 and the HA receptor RHAMM. The plastic ware was coated with poly (2-hydroxyethyl methacrylate) (poly-HEMA) to obtain a low adhesive scaffold. Cells cultured on the poly-HEMA-coated plastic ware exhibited spheroid formation without altering Bim expression. Addition of 4-MU decreased cell viability and increased Bim expression and the number of the annexin V/PI-positive (AP) cells. Moreover, exogenously applied HYAL decreased the number of spheroids and increased the number of AP cells. HA is likely necessary for spheroid formation and thus apoptosis evasion in cancer cells, suggesting that HA production could be a possible pharmacological target for tumors.