Hyaluronan-rich tumor microenvironment promotes intratumoral lymphangiogenesis.

Department of Molecular Oncology, Division of Molecular and Cellular Biology, Institute on Aging and Adaptation, Shinshu University Graduate School of Medicine; Department of Surgery, Shinshu University School of Medicine; Program of Molecular Pathology

Nobutaka Kobayashi, Hiroshi Koyama, Minoru Fujimori, Jun Amano, Reiji Kannagi, Koji Kimata, Shun'ichiro Taniguchi, and Naoki Itano

OBJECTIVE: Overproduction and accumulation of hyaluronan (HA) in the most advanced breast cancers are well documented by extensive clinical evidence. Our previous studies using the transgenic mouse model of breast cancer have shown that overproduction of HA accelerated stromal reaction accompanied by formation of intratumoral neovascularity. Using this spontaneous cancer model, we evaluated here the role of HA in tumor lymphangiogenesis.

METHODS: Mammary tumors were surgically excised from HA-overproducing and control mice. Tissue sections were then immunostained with an antibody against podoplanin, a lymphatic vessel marker. Real time quantitative RT-PCR for the gene expression analyses of vascular endothelial growth factor (VEGF)-C and -D was performed using total RNA isolated from the mammary tumors. Tumor-associated fibroblasts (TAFs) were established from mammary tumors and subcutaneously inoculated with or without TAFs into nude mice.

RESULTS: Lymphatic vessel frequently penetrated and accumulated into the stromal compartments of HA-overproducing mammary tumors. Furthermore, up-regulation of VEGF-C and -D was detected within tumor parts surrounding the stromal structures. In order to assess the contribution of stromal cells to lymphangiogenesis in vivo, we established TAFs from HA overproducing tumors, and implanted them together with human breast carcinoma cell line MCF-7 in nude mice. Carcinoma cells rapidly grew in association with marked lymphangiogenesis. Without the stromal cells, however, the tumors slowly developed with less lymphatic vessels.

CONCLUSION: Our results showed that microenvironmental HA played a pivotal role in intratumoral lymphangiogenesis. Tumor xenograft study further demonstrated significance of tumor-stromal cell cooperation in the promotion of intratumoral lymphangiogenesis.