The role of hyaluronan in tumor angiogenesis

Naoki Itano

Naoki Itano graduated from the Faculty of Science, Nagoya University and received his MS degree in 1990. He was a Research Fellow in the Clinical Research Institute, National Nagoya Hospital from 1990 to 1993 and started his professional career as Assistant Professor in the Institute for Molecular Science of Medicine, Aichi Medical University. He obtained his Ph.D. in 1997 from the Faculty of Pharmaceutical Science, Kyoto University. As a JSPS (Japan Society for the Promotion of Science) Research Fellow, he studied with Dr. Erkki Ruoslahti at Cancer Research Center, the Burnham Institute from 1999 to 2001. He was a Lecturer at Institute for Molecular Science of Medicine, Aichi Medical University from 2001 to 2005. From 2005, he has been Associate Professor in the Department of Molecular Oncology, Division of Molecular and Cellular Biology, Institute on Aging and Adaptation, Shinshu University Graduate School of Medicine. He received Young Scientist Awards of the JSCR (The Japanese Society of Carbohydrate Research) and the JAMR (The Japanese Association for Metastasis Research) in 2003.

Figure 1. Generation of Has2 conditional transgenic mice. Transgenic construct is composed of FLAG-tagged murine Has2 cDNA positioned at downstream of the transgene unit including CAG promoter (CAG Pro), a loxP sequence, the Neo-resistance gene, the SV40 poly(A) signal, and a second loxP sequence. Upon recognition of the loxP site, Cre recombinase deletes the Neo cassette along with one of the loxP sequences and then joins the CAG promoter and Has2 cDNA, leading to expression of Has2 mRNA.
Angiogenesis, the generation of new blood vessels, is an essential feature of solid tumor growth, as tumor cells require oxygen and nutrients for their proliferation and survival. The angiogenic response starts at the edge of a malignant epithelial tumor concurrently with remodeling of the microenvironment amenable to easy penetration by endothelial, inflammatory and stromal cells. Hyaluronan has been suggested to be capable of modulating blood vessel formation depending on its molecular mass. For example, high molecular weight native hyaluronan is antiangiogenic, whereas its degradation products of a specific size (3–25 disaccharide units) induce an angiogenic response.

Here we investigated the roles of hyaluronan in tumor angiogenesis using MMTV-Neu mammary tumor model. Transgenic mice that express murine hyaluronan synthase 2 (Has2) in a Cre-mediated recombination-dependent manner were generated (Fig. 1) and crossed with the MMTV-Neu mice. By expressing Cre recombinase under the control of MMTV promoter, the bigenic mice bearing Has2 and Neu transgenes exhibited a deposition of hyaluronan matrix and aggressive growth of Neu-initiated mammary tumors. As assessed by CD31 immunostaining, the number of tumor microvessels was 2-fold greater in the Has2-overexpressing mice compared with tumors of the control mice. Notably, the forced expression of Has2 also enhanced the recruitment of stromal cells into mammary tumor compared to the control mice. To reveal the molecular basis of hyaluronan-mediated neovascularization, stroma-derived growth factors and chemokines, which are known to promote an angiogenic response, were assayed for their transcriptional levels by real time quantitative RT-PCR. An almost 2-fold increase in the mRNA levels of basic FGF and SDF-1α/CXCL12 was detected in the Has2 overexpressing tumors, which is consistent with hyperneovascularization. Taken together, these results suggest that hyaluronan accelerates tumor angiogenesis through stromal cell recruitment.

**Keywords:** hyaluronan, extracellular matrix, mammary tumor, stromal reaction, angiogenesis.