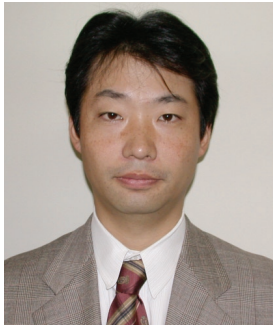




The role of hyaluronan in tumor angiogenesis

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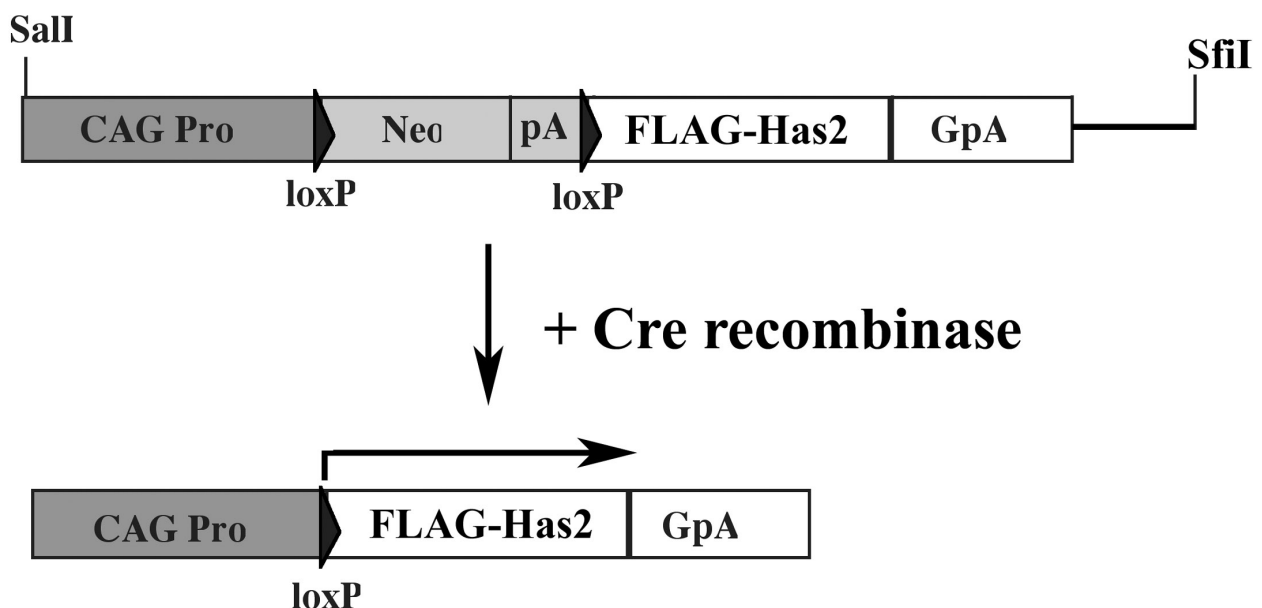
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Figure 1. Generation of Has2 conditional transgenic mice. Transgenic construct is composed of FLAG-tagged murine Has2 cDNA positioned at downstream of the transgene unit including CAG promoter (CAG Pro), a *loxP* sequence, the *Neo*-resistance gene, 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-1 α /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.