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Commentary

Hyaluronan and Tumor Growth

Bryan P. Toole* and Vincent C. Hascall†

From the Department of Anatomy and Cellular Biology, Tufts University School of Medicine, Boston, Massachusetts; and the Department of Biomedical Engineering and Orthopedic Research Center,† Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio*

The study published in this issue of *The American Journal of Pathology* by Simpson et al[1] adds a new dimension to the role of hyaluronan in cancer by demonstrating that inhibition of endogenous hyaluronan synthesis dramatically reduces tumor growth *in vivo*. In previous studies, these investigators showed that aggressive PC3M-LN4 human prostate carcinoma cells contain two of the three synthases that synthesize hyaluronan, namely HAS2 and HAS3, and that transfectants of PC3M-LN4 with antisense to HAS2 and HAS3 mRNA synthesized significantly less hyaluronan.[2,3] In the present study, stable transfectants with antisense-HAS2 and antisense-HAS3 were used alone or in combination to study tumor growth after subcutaneous injection in immunocompromised mice. The antisense-HAS transfectants produced tumors that were three to four times smaller than control tumors after 3 weeks. Although inhibition of hyaluronan synthesis reduced rates of cell proliferation in culture, the antisense-HAS transfectant tumors contained similar proportions of dividing and apoptopic cells as did the control tumors at 3 weeks. This finding suggests that the reduced size of the antisense-HAS transfectant tumors is due to reduced rates of growth early in development of the tumor. The authors also found that blood vessel density was diminished by 70 to 80%, implying that hyaluronan levels may be an important determinant of vascularity, and that this rather than proliferation is the predominant factor in the effect of hyaluronan on tumor growth in this model. Interestingly, inclusion of exogenous hyaluronan with the initial injection of the transfected tumor cells restored levels of tumor growth and vascularity to those seen in control tumors, suggesting that early angiogenic events may be crucial.

Numerous other studies have demonstrated a close correlation between tumor progression and hyaluronan production, either by tumor cells themselves or by stromal cells associated with tumors. This correlation has been observed in cell culture, in experimental animal models, and in human patients. In fact, recent work shows that hyaluronan content correlates with increased progression in several cancers, including breast, ovarian, prostate, and colorectal cancers.[4,5] In addition to the observation that hyaluronan is present in elevated amounts in numerous types of tumors, experimental manipulations of hyaluronan levels and interactions suggest a vital role for hyaluronan in promoting malignant cell behavior *in vitro* and *in vivo*. For example, experimental elevation of hyaluronan production in HT1080 human fibrosarcoma cells or TSU human prostate carcinoma cells enhances growth *in vivo.*[6,7] Also, low producers of hyaluronan selected from a population of mammary carcinoma cells are less metastatic than high producers, and metastatic capacity was restored to the low producers by elevating their hyaluronan production.[8]

Hyaluronan-Receptor Interactions

Hyaluronan interacts with several cell surface receptors, including CD44, RHAMM, LYVE-1, HARE, layilin, and Toll-4.[9,10] Hyaluronan interactions with CD44 mediate at least three important physiological processes, ie, signal transduction, assembly of pericellular matrices, and receptor-mediated internalization.[11,12] The involvement of CD44 in catabolism of hyaluronan has been shown dramatically by the failure of CD44-null tissues to clear excess hyaluronan, eg, in skin[13] and lung.[14] Inability to clear hyaluronan produced in lungs of CD44-null mice after a bleomycin inflammatory challenge results in death of the animals.[14] Polymeric endogenous hyaluronan interacts with multiple CD44 molecules on the surface of cells with consequent organization of the cytoskeleton through interactions of the cytoplasmic tail of the arrayed CD44 molecules with cytoskeleton-associated components such as ezrin and ankyrin.[11,15,16] Interference with the ability of hyaluronan to interact with the receptor interferes directly with the catabolic mechanism for hyaluronan turnover, as shown in chondrocytes[11] and keratinocytes.[17]

In similar fashion to the above, tumor growth and metastasis can also be inhibited in various animal xenograft models by perturbing endogenous hyaluronan-cell re-

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Address reprint requests to Bryan P. Toole, Department of Anatomy and Cellular Biology, Tufts University School of Medicine, Boston, MA 02111. E-mail: bryan.toole@tufts.edu.

ceptor interactions. This has been done by administering or over-expressing soluble hyaluronan-binding proteins that can bind and mask endogenous hyaluronan, by administering anti-CD44 antibodies that can displace endogenous hyaluronan from tumor cell surface CD44, or by administering small hyaluronan oligosaccharides whose monovalent interactions can disrupt the multivalent, cooperative interactions of endogenous polymeric hyaluronan with receptors such as CD44.[5,18] The use of peptide mimetics of hyaluronan that interfere with receptor binding may provide an additional approach.[19] The work of Simpson et al[1] provides another important example by interfering directly with hyaluronan synthesis by the tumor cells and therefore lowering the concentration available for interacting with the endogenous receptors.

Hyaluronan in Tumor Cell Growth and Survival

Increased hyaluronan expression or addition of exogenous hyaluronan influences several signaling pathways, including some that promote tumor cell growth and survival, eg, ErbB2, Ras, MAPK, and PI3 kinase/Akt.[10,15,16,18] The observations by Simpson et al that the antisense-HAS transfectants exhibit significantly diminished growth rates *in vitro* are consistent with this. Nevertheless, there were no apparent differences in the proportions of proliferating or apoptotic cells in tumors derived from the antisense-HAS transfectants compared to control tumors at the 3-week time point, indicating that additional mechanisms must be involved. The authors suggest that a threshold level of hyaluronan may be needed for its effects, since antisense-HAS2 or antisense-HAS3 transfection inhibited growth to the same extent as for the combined antisense-HAS2 plus antisense-HAS3 transfectant. Consequently it is possible that failure to reach a threshold level of hyaluronan may cause early changes, such as delayed growth or increased initial apoptosis that would not be detected later in tumor development. Interestingly, Rilla et al[20] have recently shown that epidermal keratinocytes stably transfected with antisense HAS2, thus having lower levels of hyaluronan synthesis, also have a significant lag in their proliferation rate *in vitro*, but reach near parental rates within a few days. The mechanism underlying this phenomenon is unknown but could conceivably lead to decreased cell mass *in vivo*.

Another possible explanation is suggested by previous studies from this group in which they showed that the hyaluronan-rich pericellular matrix around prostate tumor cells mediates interaction with bone marrow endothelial cells, and that this could provide a mechanism for tumor cell-endothelium interaction during metastasis.[2,3] Such interactions of tumor pericellular hyaluronan may contribute to "seeding" or initial survival of tumor cells during early stages of metastasis to bone or other organs. A similar conclusion was reached in another study in which inhibition of mammary tumor cell interaction with hyaluronan led to their rapid apoptosis within hours of entry into the lung interstitium.[21] Although the present study of Simpson et al does not address metastasis, similar events could regulate early stages of growth or survival after inoculation of the tumor cells, thereby leading to reduced tumor size at later times. Recent data from several laboratories[18,22,23] showing that hyaluronan is important for tumor cell growth and survival also support this idea.

It is surprising that addition of exogenous hyaluronan along with the initial inoculate of the antisense-HAS2 plus antisense-HAS3 transfectant reverted tumor growth to the control level. It might be expected that the exogenous hyaluronan would only exert its effect for a relatively short period of time before clearance and resumption of the lower levels produced by the transfected cells. This would be consistent with a mechanism that acts during the early stages of tumor development but has a major role in ultimate tumor growth, for example by activating signaling pathways that enhance cell division and migration. Other studies provide evidence that such mechanisms are likely. For example: 1) Organ cultures of the cardiac cushion endothelium from *Has2*-null mice do not undergo mesenchymal transformation and do not migrate, in contrast to wild-type cells in culture, due to aberrant Ras signaling. However, addition of small amounts of exogenous hyaluronan rescued appropriate Ras signaling, and the cells were able to transform and migrate.[24] 2) Likewise, exogenously added hyaluronan stimulates cell motility in transformed fibroblasts *via* Ras and PI3 kinase/Akt pathways.[25] These results imply that hyaluronan acts in these systems by interacting with unoccupied hyaluronan receptors or other binding proteins on the cell surface that transmit signaling information into the cell. Importantly, they also indicate that retention of hyaluronan at the cell surface by the hyaluronan synthases contributes negligibly to tumor growth. Because their previous work showed that the pericellular matrix around tumor cells promotes interaction with endothelial cells,[2,3] it would be interesting to determine whether addition of the exogenous hyaluronan caused reconstitution of a pericellular matrix around the antisense-HAS tumor cells.

Hyaluronan in Angiogenesis

Several studies have demonstrated increased angiogenesis after administration of hyaluronan oligosaccharides but inhibition of angiogenesis after administration of high molecular weight hyaluronan.[26,27] Thus, the most surprising results of this study are that inhibition of hyaluronan synthesis dramatically reduces tumor vascularity and that the co-injection of exogenous, high molecular weight hyaluronan with the inoculate of tumor cells restores blood vessel density to that of controls. Because, hyaluronan synthases produce high molecular weight hyaluronan,[28] this result appears at first glance to be contradictory to other findings. However, other studies have revealed that, in addition to hyaluronan itself, hyaluronidase levels and hyaluronan degradation correlate with tumor progression and stimulate tumor angiogenesis. It has been concluded from these studies that hyaluronidase-mediated degradation of tumor hyaluronan gener-

ates high levels of hyaluronan oligosaccharides which, in turn, stimulate tumor angiogenesis and consequently tumor growth.[29,30] This postulate is supported by a recent study indicating that increased hyaluronan expression in glioma cells that do not express hyaluronidase does not stimulate tumor progression.[31] However, hyaluronan oligosaccharides have also been shown to inhibit tumor growth *in vivo*.[32] Further studies will help to resolve these important issues.

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