Molecular Mechanism of Tenascin-C Action on Matrix Metalloproteinase-1 Invasive Potential

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**Introduction:** Chondrosarcoma is the second most common malignancy in bone and results from unregulated growth of mesenchymal stem cells with varying degrees of cartilaginous differentiation. We have shown a marked prognostic significance for recurrence in patients with chondrosarcoma high levels of matrix metalloprotease-1 (MMP-1) gene expression. Tenascin-C (TNC) is an adhesion-modulating extracellular matrix glycoprotein that is highly expressed in tumor stroma and stimulates tumor cell proliferation. We have reported that the relative expression of the large TNC splice variant may correlate with malignancy and poor clinical outcome in human chondrosarcoma. While TNC has been shown to correlate with patient survival and metastasis, the mechanism of TNC-induced metastasis has to be yet determined. Cytoplasmic signaling is induced upon cell adhesion to adhesive extracellular matrix molecules and includes activation of the mitogen-activated protein kinase (MAPK) pathway involving phosphorylation of threonine and tyrosine residues by mitogen-activated protein kinase kinase kinase. The latter is often deregulated in cancer cells. Studies have shown links between TNC and altered MAPK signaling. We hypothesized that activation of the MAPK pathway by extracellular TNC signals is involved in MMP-1 transcription. The aim of the current study was to determine if TNC220 and TNC320 differentially stimulate MMP-1 gene expression in vivo and thereby increase the invasive potential of cultured human chondrosarcoma cells. Secondary goal was to determine the involvement of MAPK in this activation process.

**Materials and Methods:** TNC320 expression vector (pNUT-Hx-B.L) and TNC220 and TNC320 proteins were gifts from Dr. Harold Erickson. Human MMP-1 promoter-driven luciferase reporter construct (nucleotides −525 to +15) and deletion constructs from the same promoter (−300/+15; −91/+15; −41/+15) were gifts from Dr. Ralf Janknecht. The chondrosarcoma cell line was cultured in a monolayer until confluent and subsequently encapsulated and recultured in alginate beads. TNC220 and TNC320 proteins were purified from a transfected CHO cell line and BHK cell line, respectively. Expression plasmids containing mitogen-activated protein kinase kinase 1 (MEK1) and mitogen-activated protein kinase kinase 1 (MEKK1) promoter with a downstream luciferase reporter construct were obtained from Clontech. All the methods described (4).

**Results:** Analysis of gene expression in cultured cells grown under different conditions (7 days) indicated a significant increase of MMP-1 mRNA expression in TNC320-treated cells compared with TNC220 (P = 0.0009) (100%) and FN and BSA. RT-PCR results demonstrate significantly higher levels of MMP-1 gene expression in TNC320-cultured cells (7 days) than in all other treatment groups, including TNC220 (P < 0.0001). The results demonstrated a 30-fold activation of MMP-1 promoter by TNC320 treatment in comparison with control and other treatments (P < 0.0001). An almost 3-fold difference in collagenase activity and invasion assays was detected in the cells pretreated with TNC320 (P < 0.01). Experiments with constitutively active expression kinases indicate that no differences in MMP-1 luc activation were observed with TNC320, FN, or BSA in the presence of kinase vectors. On the other hand, the culture with TNC320 resulted in more than 2-fold activation of MMP-1 luc activity in the presence of MEK1 and almost 2-fold down-regulation of MMP-1 luc in the presence of MEK1 (P < 0.0007). In combination, these data indicate that MMP-1 gene expression, protein levels, and invasiveness were up-regulated in cells cultured in the presence of TNC320.

**Discussion:** These data suggest that TNC320 is involved in activating the MMP-1 gene expression, protein synthesis, and possibly the initiation of extravasation. Taking into consideration our experimental results demonstrating that TNC320 up-regulates MMP-1 expression and that MAPK pathway modules are involved in this up-regulation, it may be that MAPK pathway activation is a necessary prerequisite for TNC-induced altered MMP-1 expression. This is consistent with previous studies, which provided evidence that distinct extracellular signals leading to up-regulation of MMP-1 expression involve MAPK pathways and are integrated at the level of the promoter.

**References:**

QPCR analysis demonstrates that TNC 320 stimulation of JJ012 cultured cells (7 days) significantly increased MMP_1mRNA expression compared with treatment with TNC220, FN and BSA (P=0.0009). TNC 220 generated significantly more MMP-1 mRNA than BSA alone (P=0.007).

**Human JJ012 Chondrosarcoma Cells**

Transactivation of MMP-1 luciferase construct in transiently transfected JJ012 human chondrosarcoma cultured cells (2×10^3 cells /well). Cells were seeded on top of precoated treatments of TNC 320/220 (10μg/ml), FN (2μg/ml) and BSA (1%). TNC 320 treatment causing transactivation proved to be statistically significant (P<0.0001).

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