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RAPID COMMUNICATION

Production of Endothelin-1 and Big Endothelin-1 by Human Cardiac Myxoma Cells

— Implications for the Origin of Myxomas —

Hironosuke Sakamoto, MD; Tetsuo Sakamaki, MD*; Hiroyuki Sumino, MD**; Yoshie Sawada, MD; Hiroko Sato, MD; Mahito Sato, MD; Kin’ichi Fujita, MD; Tsugiyasu Kanda, MD*; Jun’ichi Tamura, MD; Masahiko Kurabayashi, MD**

Background Although the origin of cardiac myxomas is still controversial, the 2 main hypotheses are that the tumor cells originate either from multipotential mesenchymal cells or from endocardial neural tissue.

Methods and Results The production of various cytokines in 2 human cardiac myxoma cell lines was examined by enzyme-linked immunosorbent assay. After 7 days of culture, extremely high concentrations of interleukin-6 were detected in the culture media from both myxoma cell lines. Increased production of CXC chemokines, interleukin-8 and growth-related oncogene-

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growth, angiogenesis, and malignant potential of histologically benign myxomas. Here we report the release of endothelin (ET)-1 and its precursor big ET-1 from cultured cardiac myxoma cells, supporting the hypothesis that myxoma cells originate from mesenchymal cells capable of endothelial differentiation.

Conclusions The similarity of the cytokine production pattern between cardiac myxoma cells and endothelial cells supports the hypothesis that the tumor cells originate from mesenchymal cells capable of endothelial differentiation. Overproduction of CXC chemokines may explain, in part, the malignant potential of histologically benign myxomas. (Circ J 2004; 68: 1230–1232)

Key Words: Cardiac myxoma; Endothelin-1; Growth-related oncogene-

Case Reports

Case 1
A 43-year-old woman was admitted to hospital because of aphasia and right hemiparesis. Computed tomography of the brain revealed an ischemic infarction in the territory of the left middle cerebral artery. Two-dimensional echocardiography was performed to search for a possible cardiac embolic source for the cerebral infarction and demonstrated a left atrial mass (3×3 cm) rising from the interatrial septum. The serum interleukin-6 concentration, determined by enzyme-linked immunosorbent assay (ELISA), was elevated (9.3 pg/ml). The mass was surgically removed and histologically diagnosed as a myxoma. The tumor cells in a myxoid matrix had a polygonal or spindle shape and showed a positive immunochemical reaction for interleukin-6.

Case 2
A 52-year-old woman was referred to hospital for further evaluation of T-wave inversions in leads V1-6. Two-dimensional echocardiography revealed a left atrial mass (4×3 cm) attached to the posterior wall. The serum interleukin-6 concentration was elevated (11.0 pg/ml). The tumor was completely excised and the histologic findings were consistent with a
diagnosis of myxoma. Immunohistochemistry revealed that the tumor cells reacted to IL-6 antibody.

Creation of Cell Lines and Cytokine Analysis

Human cardiac myxoma cell lines were originally isolated from the 2 different patient samples by the enzymatic digestion method as described previously. Both patients gave informed consent, and the study protocol was approved by the Gunma University Hospital ethics committee. The cell lines were maintained in 25-cm² tissue culture flasks (Costar, Cambridge, MA, USA) containing full growth medium composed of ENDO Media and TIL Media I (1:1) supplemented with 6% fetal bovine serum (all from IBL-Japan, Takasaki, Gunma, Japan). The culture media were changed weekly, and myxoma cells were subcultured monthly. Myxoma cells at passage 3 were used for the present studies.

For cytokine analysis, samples of cell culture media were obtained from each of the 2 cardiac myxoma cell lines (7.5 × 10⁶ cells) grown in 25-cm² tissue culture flasks after incubation with fresh full growth medium and 6% fetal bovine serum for 7 days. The media were stored at −80°C until the time of assay. The concentrations of human IL-6, IL-8, growth-related oncogene-α, stem cell factor, granulocyte colony-stimulating factor, hepatocyte growth factor, ET-1, big ET-1, and ET-3 were determined by ELISA (IBL-Japan). The lower limits of detection were 4.0 pg/ml for IL-6, 2.0 pg/ml for IL-8, 3.13 pg/ml for growth-related oncogene-α, 50 pg/ml for stem cell factor, 7.8 pg/ml for granulocyte colony-stimulating factor, 0.4 ng/ml for hepatocyte growth factor, and 0.76 pg/ml for ET-1, big ET-1 and ET-3.

As shown in Table 1, extremely high concentrations of IL-6 were detected in the culture media from both myxoma cell lines. Increased production of CXC chemokines, IL-8 and growth-related oncogene-α, were also detected in culture media from the same cell lines. Interestingly, the production of ET-1 and big ET-1 was detected in culture media from both cell lines. Stem cell factor, granulocyte colony-stimulating factor, hepatocyte growth factor, and ET-3 were not detected in the culture media from either cell line. We also cultured human umbilical cord vein endothelial (HUVE) cells (2.5 × 10⁶ cells), the main producers of ET-1, in a 25-cm² tissue culture flask in fresh full growth medium and 1.5% fetal bovine serum. After 7 days, the medium was collected from HUVE cells (10⁵ cells) and used for cytokine analysis. The concentrations were 62 pg/ml for growth-related oncogene-α, 88 pg/ml for ET-1, and 61 pg/ml for big ET-1, which were lower than those in the media from myxoma cells. Like myxoma cells, HUVE cells did not produce stem cell factor, granulocyte colony-stimulating factor, hepatocyte growth factor, or ET-3.

Discussion

In this study, we confirmed our previous findings that cardiac myxoma cells can produce IL-8, the prototypical member of CXC chemokines. Furthermore, for the first time we found that myxoma cells can produce growth-related oncogene-α, another member of the CXC chemokines. The production of growth-related oncogene-α by myxoma cells was higher than that by HUVE cells. Interleukin-8 has been shown to enhance angiogenesis, attract T cells, and stimulate monocyte adherence, in addition to its primary function as a chemotactic factor for neutrophils? Growth-related oncogene-α was initially identified as an autocrine growth factor for malignant melanoma cells and has subsequently been shown to serve as a neutrophil chemotactic and angiogenic factor as well. Overexpression of IL-8 and growth-related oncogene-α has been shown to promote tumor growth and metastasis. Although cardiac myxomas usually present as a benign neoplasm, there are many reports suggesting its malignant potential, including recurrence of tumor, locally invasive myxoma, extension from the heart, and distant metastasis. Overproduction of IL-8 and growth-related oncogene-α by myxoma cells may explain the malignant potential of this tumor.

To the best of our knowledge, this is the first report showing that cardiac myxoma cells constitutively produce large amounts of ET-1 and big ET-1. ET-1 is a 21-amino-acid peptide that has potent vasoconstrictor activity. Active mature ET-1 is generated from the 38-amino-acid inactive precursor big ET-1 by catalysis by an ET-converting enzyme. ET-1 seems to contribute to the development of cardiovascular diseases, and modulates vascular tone and blood flow, as well as promoting vascular cell growth in an autocrine or paracrine fashion through 2 subtypes of receptors. Two other isoforms of ET have been discovered (ET-2 and ET-3), but vascular endothelial cells predominantly produce ET-1. Whether overproduction of ET-1 plays a physiopathological role in cardiac myxomas is unknown. However, our results, in conjunction with previously published results, support the hypothesis that myxoma cells originate from mesenchymal cells capable of endothelial differentiation. Other cytokines, including IL-6, IL-8, monocyte chemotactic protein-1, and growth-related oncogene-α, are expressed after induction of endothelial cells with various cytokines. A recent report provides in-vitro evidence of autocrine secretion of vascular endothelial growth factor by endothelial cells from human placental blood vessels. Thus, the cytokine expression pattern in myxoma cells is similar to that in HUVE cells. Furthermore, the vasculogenic tendency of myxoma cells strengthens the hypothesis. The presence of primitive mesenchymal cells in the interatrial septum is still mysterious, and the search for mesenchymal cells differentiating into myxoma cells should be continued.

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References


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<th>Case 2</th>
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<td>77,900</td>
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<tr>
<td>Interleukin-8 (pg/ml)</td>
<td>300</td>
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<tr>
<td>Growth-related oncogene-α (pg/ml)</td>
<td>345</td>
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<td>Endothelin-1 (pg/ml)</td>
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<td>Big endothelin-1 (pg/ml)</td>
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<td>Endothelin-3 (pg/ml)</td>
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