Expression Of Her Family And Effect Of HER2 Inhibitor In Soft Tissue Sarcoma

Hironori Date[1], Kunihiro Asanuma[2], Hiroto Yuasa[3], Yumi Matsuyama[1], Tomohito Hagi[1], Tomoki Nakamura[1], Masahiro Hasegawa[1]
[1] Musculoskeletal Surgery・Multimodality Therapy for Cancer (Orthopaedic Surgery), Graduate School of Medicine, Mie University, Tsu, Mie, Japan
[2] Department of Orthopaedic Surgery, Matusaka Chuo General Hospital, Matusaka, Mie, Japan
[3]Department of Oncologic Pathology and Diagnostic Pathology, Mie University, Tsu, Mie, Japan

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INTRODUCTION: We report a case of Li-Fraumeni syndrome with synchronous triple cancer (breast cancer, brain glioma, and leiomyosarcoma) in which trastuzumab and pertuzumab combination therapy resulted in tumor shrinkage of the leiomyosarcoma in a patient with HER2-positive breast cancer. Therefore, we investigated the expression of the HER family in soft tissue sarcomas at our institution and the efficacy of HER2 inhibitors in soft tissue sarcomas (human synovial sarcoma: HS-SY-2).

METHODS: The study included 51 cases of malignant soft tissue tumors (34 males, 17 females) diagnosed between 1998 and 2017. The median age was 55.2 years, the mean survival period was 101 months, and the 5-year survival rate was 63.9%. Immunohistochemical staining for HER2/3/4 was performed on resected specimens. HER2 expression was assessed by membrane staining intensity and percentage of stained cells, while HER3/4 expression was evaluated by nuclear staining intensity (0–3 points) and percentage of stained cells, as well as cytoplasmic staining intensity (0–3 points) and percentage of stained cells, all determined by a pathologist. Correlations between HER staining results and recurrence, metastasis, and prognosis were evaluated using the log-rank test.

Human synovial sarcoma: HS-SY-2 was subjected to the same HER2/3/4 immunohistochemical staining and Western blotting to evaluate expression.

In vitro: The effect of pertuzumab on cancer cell proliferation was investigated using human synovial sarcoma: HS-SY-2. Cell lines (1×10^4/well) were seeded into a 96-well plate, cultured for 24 hours, then replaced with serum-free medium and incubated for 2 hours.Pertuzumab (concentration: 100 nmol/L) was added and incubated for 2 hours, followed by addition of rHRGβ1 (concentration: 5 nmol/L), the ligand for HER3, and cultured for 24 hours before harvesting and evaluating by MST assay.

To evaluate MAPK phosphorylation, cells (20×10^4/well) were cultured in serum-containing medium in a 6-well plate. After 24 hours, the medium was removed and fresh medium containing 0.1% serum was added.

After overnight culture, the medium was replaced with serum-free medium and incubated for 3 hours. The cells were then incubated with 1 ml of 100 nM pertuzumab for 1 hour. Subsequently, the cells were treated with 5 nM HRGβ1 for 15 minutes, and p-MAPK (Erk1/2) was evaluated by Western blotting.

In vivo: HS-SY-2-transplanted nude mice (BALB/c nu/nu, 5 weeks old, male with tumor diameter ≥5 mm
n=4) were administered pertuzumab at 1.2/0.6 mg/kg intravenously for 7 weeks, totaling 7 doses. Tumor diameter was measured twice weekly, and animals were sacrificed on day 49. Tumor diameter was calculated as tumor volume= longest diameter × {(shortest diameter)2/2}, Plot the volume ratio from day 0 and significant differences were evaluated using the Mann-Whitney U test.

RESULTS SECTION: Staining results: The cases included 17 synovial sarcomas (SS), 12 undifferentiated pleomorphic sarcomas (UPS), 10 malignant peripheral nerve sheath tumors (MPNST), 7 leiomyosarcomas (LS), 3 myxoid fibrosarcomas (MS), and 2 other cases.The proportions of cases showing HER staining (HER2 cell membrane, HER3 nucleus, cytoplasm, HER4 nucleus, cytoplasm) were 51%, 29%, 25%, 53%, and 53%, respectively. No cell membrane expression of HER3 or HER4 was observed. By tissue type, the proportions were SS (82, 29, 18, 59, 71)(Figure1),UPS (33, 42, 33, 25, 33), MPNST (40, 50, 30, 80, 60), LS (29, 0, 14, 43, 14), MS (67, 0, 67, 33, 100), and others (0, 0, 0, 100, 50).HER2 expression showed no significant differences in recurrence, metastasis, or prognosis. A nuclear staining rate of 30% or higher for HER3 was significantly associated with increased recurrence ($p=0.0367$), but no significant differences were observed in metastasis or prognosis.HER4 expression did not differ in recurrence, but there was a trend toward increased metastasis with nuclear intensity of 2 or higher ($P=0.0573$) and significantly poorer prognosis ($p=0.0322$). Cytoplasmic expression did not correlate with recurrence, metastasis, or prognosis.

Further evaluation was conducted on synovial sarcomas with high HER2 positivity. Human synovial sarcoma: HS-SY-2 was evaluated for HER2/3/4 expression using the same methods as above, including immunohistochemistry and Western blotting.

Immunohistochemistry revealed expression of HER2/3/4. Western blotting detected bands for HER2/4. Therefore, human synovial sarcoma: HS-SY-2 was determined to be a HER2/3/4-positive cell line.

In vitro: Cell proliferation was significantly higher in the HRG-treated group compared to the control group ($p=0.00952$).When pertuzumab was administered to HER2-positive human synovial sarcoma HS-ST-2, it inhibited cancer cell proliferation compared to HRG stimulation. Compared to the control group, the pertuzumab-treated group showed significantly suppressed cell proliferation ($p = 0.0317$). Western blotting revealed a reduction in the p-MAPK (Erk1/2) band following pertuzumab administration(Figure2).

In vivo: In HS-SY-2-transplanted mice (BALB/c nu/nu, 5 weeks old, male, n=4), pertuzumab administration resulted in tumor growth inhibition compared to the non-treated group, with significant reduction observed from day 17 onwards ($p=0.0317$)(Figure3).

DISCUSSION: The leiomyosarcoma cases we experienced were HER2-negative, HER3-nuclear-positive, and HER4-nuclear-positive, but showed a reduction effect with the combination therapy of trastuzumab and pertuzumab.Pertuzumab has a synergistic inhibitory effect on HER2, HER3, and HER4. In a study of 51 cases of malignant soft tissue tumors, HER family expression was high in HER2, HER4 nuclear, and cytoplasmic expression, with a significant correlation between HER4 nuclear expression intensity and prognosis. This suggests that inhibition of HER4 may be effective against sarcomas.In vitro experiments using HER2-positive human synovial sarcoma (HS-SY-2) cells demonstrated tumor proliferation inhibitory effects of pertuzumab under HRG stimulation alone. Additionally, Western blotting revealed a reduction in the p-MAPK (Erk1/2) band following pertuzumab administration. This suggests that, similar to breast cancer, in soft tissue sarcomas (synovial sarcomas), pertuzumab administration may weaken signal transduction in the MAPK pathway and inhibit tumor proliferation in HER2-positive cases.In vivo, tumor growth was significantly inhibited compared to the control group. Based on the above, pertuzumab was evaluated as having a certain effect in HER2-positive synovial sarcoma.

SIGNIFICANCE/CLINICAL RELEVANCE: This study demonstrated a certain efficacy of pertuzumab in HER2-positive synovial sarcoma. We anticipate that this finding may contribute to the development of molecularly targeted therapy for soft tissue sarcomas in the future.

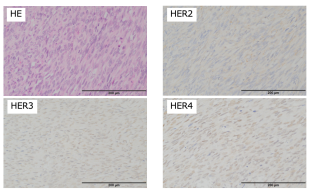

Figure1. HER2/3/4 immunohistochemistry Synovial sarcoma

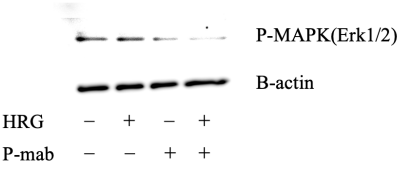

Figure2. P-MAPK (Erk1/2) Western blotting

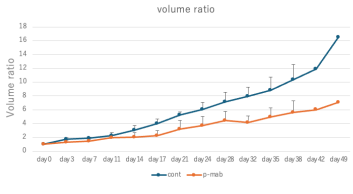

Figure3. Tumor volume ratio changes