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Erdheim-Chester Disease: Report of a Case with PCR-based Analysis of the Expression of Osteopontin and Survivin in Xanthogranulomas Following Glucocorticoid Treatment

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Abstract. Erdheim-Chester disease (ECD) is a form of non-Langerhans histiocytosis. In this report, we show a case of ECD presenting diabetes insipidus and multiple xanthogranulomas received glucocorticoid treatment over a year. During this period, xanthogranulomas improved in response to the glucocorticoid therapy. Furthermore, the expression of osteopontin in xanthogranulomatous tissues significantly decreased following the treatment. Our data show the expression of osteopontin in xanthogranulomatous tissues of ECD. Furthermore, the osteopontin mRNA decreased following glucocorticoid therapy with xanthogranuloma regression, suggesting that the expression level of osteopontin could be a marker of the disease activity of ECD.

Key words: Erdheim-Chester disease, Osteopontin, Survivin, Xanthogranuloma, Diabetes insipidus, Non-Langerhans histiocytosis

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ERDHEIM-CHESTER disease (ECD) is a form of non-Langerhans histiocytosis characterized by xanthogranulomatous infiltration of foamy macrophages [1]. Only 250 cases have been reported in the literature [2]. The symptoms and clinical manifestations depend upon the organ(s) involved. The common sites involved are soft tissues, skin, long bone, lung, heart, aorta and the central nervous system including the pituitary gland [3, 4]. Infiltration of the pituitary stalk sometimes leads to diabetes insipidus, and infiltration of the lung may lead to diffuse pulmonary fibrosis and cardiorespiratory failure [5, 6]. The diagnosis is based on specific pathological findings or radiographical changes such as bilateral osteosclerosis, which involves mainly the metaphyses of the long bones [7]. The prognosis is poor and often fatal, with death due to cardiomyopathy, respiratory or renal failure [8].

The pathogenesis of ECD has not been clarified to date, and no standard therapeutic strategies have been established. Immunosuppressive agents such as glucocorticoids, methotrexate, and cyclophosphamide have been used for some patients with ECD [9–11], but their effectiveness is controversial. One of the obstacles in evaluating the effect of the drugs is that there are no good markers reflecting the activity of the disease. Recently, two substances have been reported to be overexpressed in either reactive/inflammatory or neoplastic tissues. One of these substances is osteopontin (OPN), which is a secreted adhesive, glycosylated phosphoprotein, which contains an arginine-glycine-aspartic acid cell-binding sequence that is found in many extracellular matrix proteins [12]. The other substance is survivin, which is one of the inhibitors of apoptosis proteins (IAPs) overexpressed in a variety of tumors [13] but not in normal tissues [14].

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In this report, we show a case of ECD in a 27-year-old woman presenting multiple xanthogranulomas with diabetes insipidus. In this case, we have quantitatively examined OPN and survivin mRNA in xanthogranulomas during the course of glucocorticoid treatment, in order to confirm our speculation that the expression levels of these two markers are related to the pathogenesis and/or the disease activity of ECD.

Case Report

A 27-year-old Japanese woman was referred to our hospital in 2002 with a complaint of xanthogranulomas (Fig. 1A), fever of unknown origin, and bone pain. The patient had histories of bronchial asthma and atopic dermatitis in her childhood. Three years before admission, she experienced fever (37.0–38.5°C), polyuria and polydipsia. She was admitted to another hospital and had magnetic resonance imaging (MRI) of the brain, which revealed an enlarged pituitary gland with a thick stalk (Fig. 1B). She was thus suspected to have a pituitary tumor and received transsphenoidal surgery, with subsequent histological diagnosis of inflammatory tissue of unknown etiology. After surgery, slowly-progressive xanthogranulomas developed in her eyelids, postauricular, and precordial regions (Fig. 1A), and she was referred to our hospital for further examination. On admission, she was febrile (38.0°C), but her blood pressure (120/72 mmHg) and pulse rate (70/min) were normal. Soft yellow linear and confluent brownish papules and nodules were observed on the eyelids, postauricular and precordial regions, and both axilla. No lymphadenopathy was detected. Laboratory examinations showed that her urinalysis, complete blood counts, routine blood chemistry, lipid profile, and immunoelectrophoresis were all normal. Basal values of thyroid hormones and anterior pituitary hormones were also normal. Serum osmolality (289 mOsm/kg) stayed within the physiological range under the treatment of dDAVP nasal spray. Tumor markers including CEA, CA15-3 and CA19-9 were all within the normal limits. Plasma cytokines (TNF-α, M-CSF and IL-1β were not elevated (data not shown), but serum soluble IL-2 receptor and plasma IL-6 were both slightly elevated; 1450 U/ml (normal<530 U/ml) and 4.4 pg/ml (normal<4.0 pg/ml), respectively.

A CT scan of the patient's thorax showed mass lesions in both axilla and mammary glands (Fig. 1C), suggesting the possible existence of xanthogranulomas. A brain MRI showed postoperative changes of the hypothalamo-pituitary region with a slightly thick pituitary stalk (see below). A bone scan analysis showed a symmetrical increase of radioisotope uptake in her shoulders, elbows, distal radii and ulnae, distal femurs, and tibia condyles (Fig. 1D). In addition, MRI analysis of the spine and both knees showed a low heterogeneous signal on T1-weighted sequences and a low signal on T2-weighted sequences in the spine, bilateral distal femurs, tibias and fibulas (Fig. 1E and 2A).

The patient received a biopsy for the xanthogranulomas of eyelids (Fig. 1F), and the subsequent immunohistochemical examination showed foamy macrophages positive for CD68 (Fig. 1G) and negative for S-100 protein (data not shown). Electron microscopic examination of the same biopsy specimen showed no Birbeck granules. Bone biopsy of her tibial area also displayed foamy lipid laden macrophages as xanthogranulomas. Based on the combined clinical and pathological findings, we diagnosed the patient's primary disease as ECD.

For the treatment of the patient's ECD, she initially received steroid pulse therapy (methylprednisolone 1 g/day for 3 days), followed by daily oral administration of prednisolone (PSL, 60 mg/day to 10 mg/day) for a year. During that period, she experienced no exacerbation of the symptoms, and repeated CT scan analysis revealed the improvement of her xanthogranuloma of the mammary gland (Fig. 2). The findings of her pituitary gland, spine and femur bone MRI also indicated no progression of the disease during the glucocorticoid treatment (Fig. 2).

Materials and Methods

Sample preparation

Biopsy specimens were obtained from xanthogranulomas of precordial region, auricula, and eyelids when the patient had the operation to remove the xanthogranulomas for cosmetic purposes. Before undergoing genetic analysis, written informed consent was obtained from the patient.

Conventional RT-PCR

The expression of OPN and survivin mRNA in xan-

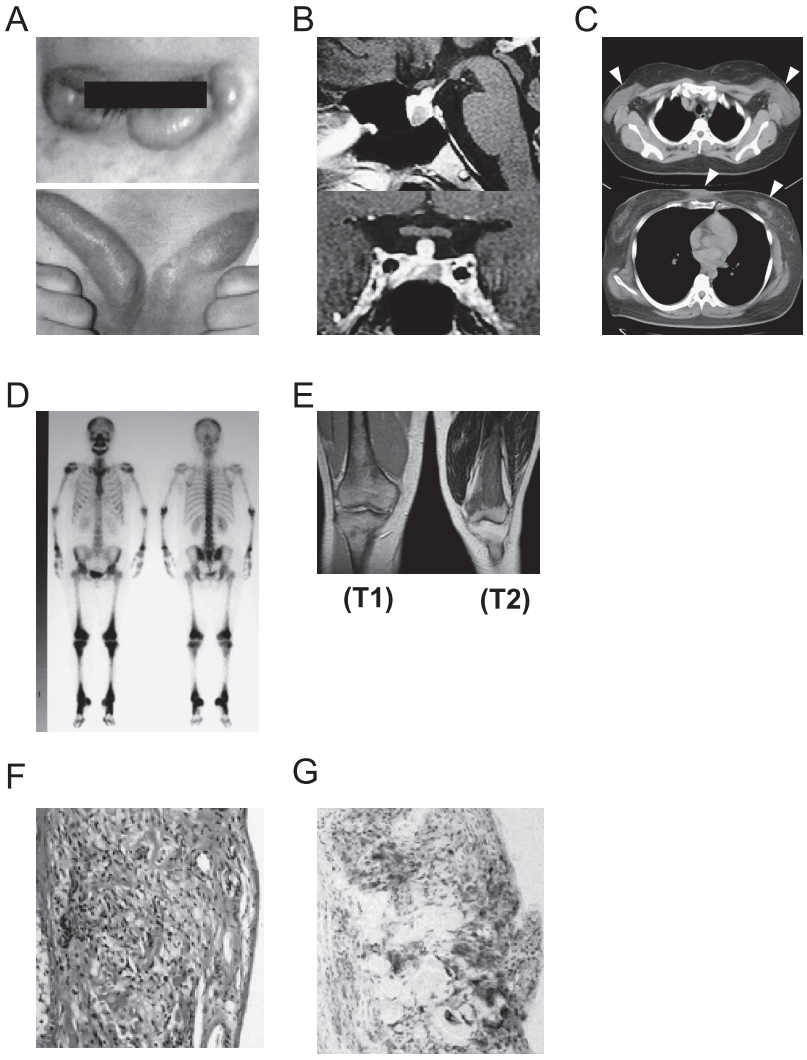

Fig. 1. (A) Photographs of xanthogranulomas at the right eyelid (upper panel) and precordial region (lower panel). (B) MR imaging of the pituitary region before surgery and glucocorticoid therapy. Photographs represent T1-weighted images following gadolinium enhancement by sagittal (upper panel) and coronal (lower panel) scan. (C) CT imaging of the chest showing xanthogranulomatous lesions at the bilateral axilla, left mammary gland and precordial regions (*arrows*). (D) bone scan. The photograph indicates increased uptake at metaphyseal regions of the long bones. (E) MR imaging of the knee joints. Photographs represent a T1-weighted coronal image of the right knee joint (left panel) and T2-weighted coronal image of the left knee joint (right panel), both showing a medullary heterogeneity predominantly in the femur. (F, G) Histological findings of the xanthogranulomas of eyelids. (F) Hematoxylin and eosin staining (×100). (G) Immunohistochemistry for CD68 (×100). The photograph shows foamy histiocytes stained strongly positive for CD68 in xanthogranulomas.

thogranulomas of the patient was examined by conventional RT-PCR. Total RNA was isolated from the patient's biopsy specimens using an RNeasy RNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The cDNA was then synthesized with Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA) using 2 μg of total RNA, and PCR was carried out under standard amplification conditions. The primer sets used were: human OPN, forward 5'-ATCACCTGTGCCATACCAGTTAAAC-3', reverse 5'-CCACAGCATCTGGGTATTTG-3'; human survivin, forward 5'-GGGCTGCCACGTCCAC-3', reverse 5'-GTCGTCATCTGGCTCCCA-3'.

Quantitative real-time RT-PCR

For quantitative estimation of OPN and survivin mRNA before and after glucocorticoid treatment, we

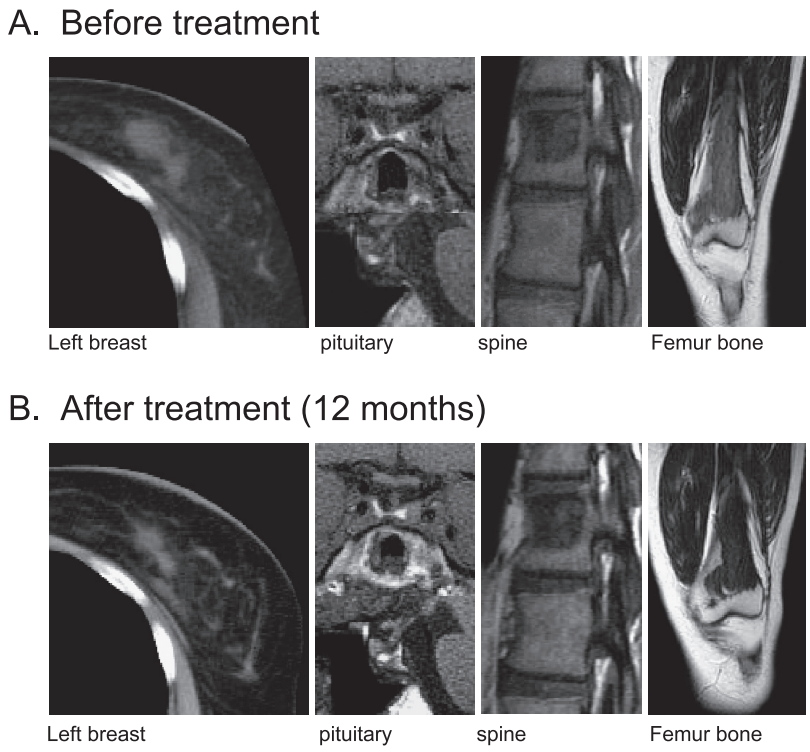

Fig. 2. Photographs of MR and CT imaging before (A) and after (B) glucocorticoid treatment for ECD. CT imaging of the left breast shows a significant reduction in xanthogranulomas after glucocorticoid treatment (left panels). MR imaging of the pituitary (T1-weighted coronal and sagittal), spine (T1-weighted sagittal), and femur bone (T2-weighted coronal) shows no progression of the xanthogranulomas following glucocorticoid treatment. Note that the patient had transsphenoidal surgery for removing a pituitary mass and glucocorticoid administration for bronchial asthma before the current glucocorticoid treatment.

also carried out real-time RT-PCR analysis. Total RNA was isolated from the xanthogranulomas of peripheral parts of eyelids, and cDNAs were synthesized, as described above. Reaction mixture for TaqMan real-time PCR was prepared according to the manufacturer's instructions [10 μM each of forward and reverse primers, 10 μM TaqMan probe, 12.5 μl TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA), H_2O and 2 μl of cDNA mixture, in a total volume of 25 μl]. TaqMan probes used were: OPN, 5'-TAAAGCTGCTTTTCCTCAGAACTTCCAGAATCA-3', and human survivin, 5'-TTCATCCACTGCCCCA CTGAGAACGA-3' [15, 16]. Forward and reverse primer sets for human OPN and survivin were the same as conventional RT-PCR (see above). After the enzyme activation (2 min at 50°C and 10 min at 95°C), cycle amplification for each reaction mixture (45 cycles) was performed (15 sec at 95°C and 60 sec at 60°C) by an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) (n = 4). Finally, the amount of each mRNA was quantified, using human GAPDH mRNA as an internal control.

Data analysis

All data are expressed as the mean ± SE. When the statistical analyses were performed, data were analyzed by one-way ANOVA with Fisher's protected least squares difference test, and $P<0.05$ was considered significant.

Results

We first examined the expression of OPN and survivin in the patient's xanthogranulomas from the precordial region, auricula, and eyelids by conventional RT-PCR, and showed the presence of both mRNAs (Fig. 3A). We also examined the quantitative analysis of OPN and survivin mRNA in xanthogranulomas using the real-time RT-PCR technique before and after the glucocorticoid treatment. We found that PSL ad-

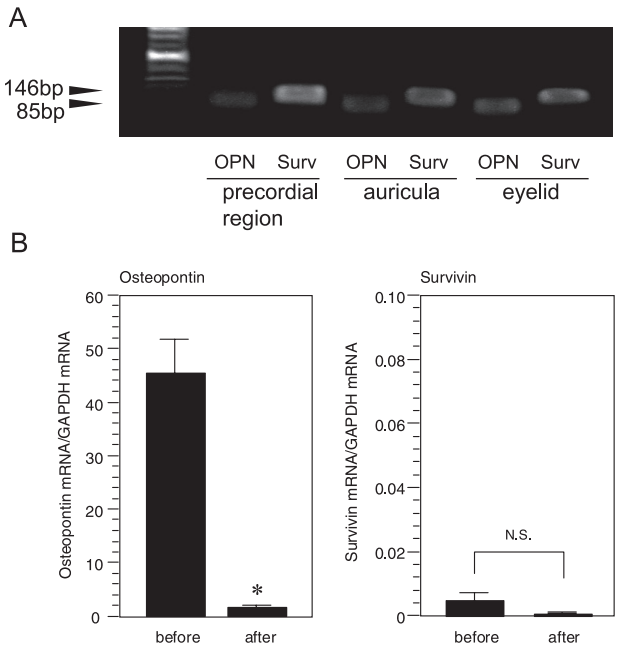

Fig. 3. (A) Expression of OPN and survivin (Surv) mRNA in xanthogranulomas from the eyelid, auricula and precordial regions. Total RNA was extracted from the biopsy specimen of the xanthogranulomas, and analyzed by conventional RT-PCR. (B) Quantitative analysis of OPN and survivin mRNA before and after glucocorticoid treatment by real-time RT-PCR. GAPDH mRNA was used as an internal control. Total RNA was isolated from the xanthogranulomas of peripheral parts of eyelids. *P<0.05 vs. before treatment.

ministration for a year significantly decreased the expression levels of OPN mRNA in xanthogranulomas (Fig. 3B). Expression levels of survivin mRNA were extremely low before treatment (when GAPDH mRNA was used as an internal control), and appeared to be suppressed after glucocorticoid treatment, although it did not reach statistical significance.

Discussion

The clinical characteristics and radiographic findings in this case are all consistent with ECD. Veyssier-Belot *et al.* [17] reported a retrospective evaluation of clinical features of 59 ECD patients, and showed that the most frequent clinical sign was bone pain (28/59), followed by polyuria due to diabetes insipidus (17/59) and xanthogranulomas (11/59) (mainly located in the eyelids). The present case in this report also had the symptoms described above. The pathological and immunohistochemical findings of the biopsy specimens in this case also support the final diagnosis of ECD. In endocrinologic disease, pituitary tumor, diabetes insipidus and xanthogranulomas are important clinical findings that should be taken into account to rule out ECD.

The pathogenesis of ECD is not completely understood. Chetritt *et al.* [1] and Vencio *et al.* [18] revealed the monoclonal origin of xanthogranulomatous cells, suggesting that the disorder is neoplastic rather than reactive in nature. Alternatively, Gotthardt *et al.* [19] suggested that ECD is a lipid storage disorder. In this report, we examined the expression of two biological markers, OPN and survivin, in xanthogranulomas using the RT-PCR technique. OPN is a non-collagenous extracellular matrix protein with pleiotropic functions [20], and plays a variety of important roles in cell adhesion, migration, calcification, immunity [21], monocyte infiltration [22] and atherosclerosis [23]. Interestingly, OPN is expressed in bone (especially in the metaphyseal and periosteal bone) [24], pituitary [25], mammary glands [26], kidney and lung [27], the distribution spectrum of which is quite similar to those of xanthogranulomas in ECD, where xanthogranulomatous infiltration of macrophages occurs. Furthermore, in necrobiotic xanthogranuloma, macrophages expressing OPN are reported to contribute to the intracellular accumulation of lipoprotein-derived lipids leading to non-inherited xanthomatosis [28]. Thus, we speculated that the expression of OPN could be a marker of the disease activity of ECD. The results obtained in this study showed that although mRNA of both proteins was detected by conventional RT-PCR, the expression levels of OPN in xanthogranulomas were significantly decreased by the PSL administration for a year. Expression levels of survivin mRNA were extremely low and not significant between before and after the PSL treatment. This suggests that the cells in xanthogranulomas of ECD are not highly neoplastic in nature, although the concept of ECD as a neoplastic disease cannot be entirely excluded because OPN has also been recently reported to be overexpressed in tumor cells [29]. Besides the nature of ECD, our data suggest that OPN rather than survivin may be applicable as a marker of disease activity.

The clinical therapeutic strategy of ECD has yet to be established. Some reports have shown the effectiveness of immunosuppressants such as glucocorticoids [11], methotrexate [9], and cyclophosphamide, whereas others have shown that vinblastine, adriamycin [10],

interferon [2] and radiotherapy [30] are also effective. In our case, glucocorticoid treatment (initial pulse therapy followed by oral administration of prednisolone for a year) improved the xanthogranuloma of the mammary gland, and also prevented the disease progression in other tissues. More importantly, the expression levels of OPN in xanthogranulomatous cells were markedly decreased after glucocorticoid treatment. Considering that the natural course of ECD is progressive with poor prognosis, our findings, both clinical and basic, strongly suggest that glucocorticoid therapy may be effective for slowing down the progression of the disease. Indeed, the patient received glucocorticoid administration for her bronchial asthma in other hospitals when she was 25 years old, and during that time pituitary stalk thickness by MRI examination was partially diminished (Fig. 1B and 2A).

In summary, we found that OPN is expressed in xanthogranulomas of ECD and may be useful as a marker of the disease activity. Although the expression of OPN may somehow be related to the pathogenesis of the disease, further examination is needed to clarify the issue. Finally, our data suggest that glucocorticoid treatment is effective in some cases of ECD, for the improvement of the clinical symptoms and suppression of the disease progression.

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