Clinicopathological value of ErbB2 gene and protein expression in osteochondroma

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ABSTRACT

Objective: The aim of this study was to investigate ErbB2 expression in osteochondroma and its relationship with clinicopathologic features of osteochondroma, so as to identify a new biomarker for the malignant transformation potential of osteochondroma.

Methods: Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) were used to investigate the expression status of ErbB2 protein and gene in 30 osteochondroma tissues and 20 non-neoplastic bone tissues. The association of ErbB2 gene and protein expression with clinicopathological parameters of osteochondroma was analyzed by using the χ² test and Fishers exact test.

Results: ErbB2 protein was found to be over-expressed in 4 of 30 (13.3%) osteochondromas and 1 of 20 (5%) non-neoplastic bone samples, which were not statistically significant (p=0.336). However, 13 of the 30 (43.3%) osteochondromas showed ErbB2 gene amplification, which was not found to be significant in any of the non-neoplastic bone tissue. ErbB2 gene amplification in osteochondroma was significantly higher compared with that in non-neoplastic bone tissue (p=0.001). In addition, the ErbB2 gene amplification was closely associated with clinicopathological parameters of osteochondroma, including high expression of cellularity (p=0.001), presence of binucleated cells (p=0.001), nuclear pleomorphism (p=0.003), calcification (p=0.002), nodularity (p=0.002), necrosis (p=0.009) and cartilage thickness (p=0.026). The association of the gene amplification with other clinicopathological parameters of osteochondroma included permeation of trabecular bone, cystic/mucoid changes, mitosis, radiographic appearance, cap volume and subtype of osteochondroma was not observed. The over-expression of ErbB2 protein was not found to be associated with the above stated clinical pathological parameters of osteochondroma.

Conclusion: ErbB2 gene amplification was associated with adverse clinicopathological status of osteochondroma and could serve as an index for malignant conversion of osteochondroma. Further research is required to verify the predictive values of ErbB2 for osteochondroma.

Level of Evidence: Level IV, Diagnostic Study

Osteochondroma is the most common benign tumor of bone tissues and may present either as a solitary lesion or multiple lesions (1), generally located on the metaphysis of younger patients, especially those of the tibia, femur, and humerus (2). Radiological evaluation with X-ray or MRI is an efficient method for osteochondroma diagnosis (3). Although most patients with osteochondroma feel no symptom in the disease course, sufferers with a lesion in the proximal tibia are frequently symptomatic due to abundant tendinous structures and lack of soft tissue. For patients with complications of pain, tendon irritation, overlying bursitis, nerve compression, joint motion limitation, growth disturbance, or malignant transformation, surgery is recommended (4). Malignant transformation of osteochondroma into secondary chondrosarcoma, which is estimated to occur in 0.5–5% of patients (5), is the most serious complication of osteochondroma. Patients with secondary chondrosarcomas undergo diverse clinical courses, with a range from slow insidious tumor growth to rapid neoplastic progression, especially for tumors located in shoulder, pelvis and hip (6). Therefore, investigation of molecular markers for the malignant conversion tendency of osteochondroma is of significance.

As a member of the tyrosine kinase receptor, the ErbB2 gene encodes human epidermal growth factor receptor 2, a 185-kD transmembrane glycoprotein with intrinsic tyrosine kinase activity (7). ErbB2 is linked to poor prognosis in numerous epithelial and mesenchymal tumors (8). The ErbB2 gene was found to be amplified in 15%–40%
of breast cancers, and was associated with poor disease-free and overall survival of patients (9). One study by Liu et al. demonstrated that ErbB2 overexpression was associated with high risk of tumor metastasis and worse survival in patients with osteosarcoma (10). In synovial sarcoma, ErbB2 protein overexpression plays an important role in tumorigenesis, and ErbB2-targeted antibody therapy may provide potential efficacy for soft-tissue tumors (11). A recent phase I/II clinical study showed well the safety and efficacy of ErbB2-specific immunotherapy for metastatic or recurrent sarcomas (12).

The role of ErbB2 in osteochondroma has not been reported yet. The aim of our study was therefore to investigate the expression status of ErbB2 gene and protein in osteochondroma and non-neoplastic bone tissue by applying immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) methods. The relationship of ErbB2 protein and gene expression with clinicopathological features of osteochondroma was also analyzed, so as to identify a new prognostic biomarker for osteochondroma.

Materials and Methods

A total of 30 osteochondroma patients who underwent surgical resection were selected retrospectively from the Department of Orthopedics at the First Affiliated Hospital of Fujian Medical University (Fuzhou, China) between January 1, 2016, and June 30, 2018. All the patients included were diagnosed with osteochondroma by pathological examination. Clinicopathological data, including age, sex, tumor size, subtype, tumor location, presence of cellularity, binucleated cells, nuclear pleomorphism, calcification, nodularity, permeation of trabecular bone, cystic/mucoid changes, necrosis, mitosis, radiographic appearance, cartilage thickness, and cap volume were retrieved. The thirty formalin-fixed, paraffin-embedded tumor samples were obtained from the archives of the Department of Pathology for immunohistochemical staining and FISH, with 20 corresponding non-neoplastic bone tissues which were resected between 2016 and 2018 used as controls. After surgical resection, each patient was monitored under X-ray or CT scans in the primary lesion area every 3 months in the 3 years following in order to evaluate recurrence status.

All participants involved in our study provided written informed consent. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the Ethics Committee in the First Affiliated Hospital of Fujian Medical University (Fuzhou, China) with file number [2015]041.

Immunohistochemistry (IHC)

Fifty archival specimens received immunohistochemical staining using the PV9000 immunohistochemical kit (Origene Technologies, Inc., Beijing, China). All specimens were fixed in 10% formalin for 24–48 h at room temperature, embedded in paraffin, and serially sectioned with 4 µm thickness. After incubation at 60 °C for 1 h, the tissues were deparaffinized, dehydrated, and incubated with 3% hydrogen peroxide for 10 min at room temperature to block endogenous peroxidase activity. During the antigen retrieval process, the sections were microwaved in citrate buffer (pH 6.0) for 2 min and then passively cooled to room temperature. Subsequently, the sections were incubated with anti-ErbB2 antibody (rabbit monoclonal antibody EP1045Y; Abcam, cat.no. ab134182; diluted 1:100) at 37°C for 1–2 h, and then with poly peroxidase-anti-rabbit IgG (Origene Technologies Inc.) for 20 min at room temperature. In the following stage, the sections were stained with dianobenzidine (Origene Technologies, Inc.) for 3–5 min and counterstained with hematoxylin for 2 min at room temperature, then dehydrated and mounted. Negative (phosphate-buffered saline rather than primary antibody) and known positive (breast carcinoma tissue) controls were stained in parallel with the sections. Additionally, for all specimens hematoxylin-eosin (H&E) staining was used to identify the tumor site.

The staining results were independently observed and interpreted by two pathologists. Brown particles in cytoplasm or membrane were considered ErbB2-positive staining. Expression was graded according to the staining intensity of tumor cells from 0 to 3 as follows: 0, negative; 1, weakly positive; 2, moderately positive; 3, strongly positive. The mean percentage of staining of positive tumor cells was scored from 0 to 3 as follows: 1, <25%; 2, 25%–75%; 3, >75%. The final metric calculated by the product of the density and percentage of positive-staining tumor cells included scores of 0, 1, 2, 3, 4, 6 and 9. Scores >2 were defined as over-expression, and scores ≤2 were considered low-expression (13).

Fluorescence in situ hybridization (FISH)

ErbB2 gene amplification detection using the Her2/CEP17 Dual Color FISH Probes kit (cat.no. BFGO-AD02-10, AmoyDx, Xiamen, China) was performed according to the manufacturer’s instructions. After incubation at 56 °C overnight, the 4 µm thick slide specimens were deparaffinized, dehydrated, incubated in boiling deionized water for 20 min, and then digested with protein K at 37 °C for 20 min. The Her2/CEP17 probe was denatured at 85°C for 5 min before hybridization. Sections were incubated with the probe overnight at 37°C in a humidified chamber. Subsequent to post-hybridization washes, sections were stained with 4',6-diamidino-2-phenylinodole (DAPI) and mounted. Lesions evident on H&E-stained slides were compared with those on DAPI-counterstained FISH slides to ensure that the lesions of interest were evaluated. Finally, the sections were observed under a fluorescence microscope (Olympus, Tokyo, Japan).

For each section, 50 consecutive, well-visualized, non-overlapping epithelial nuclei under the fluorescence microscope were analyzed to calculate the Ratio value (Ratio value=total number of red signals/green signals in the nucleus). The results were evaluated by two pathologists independently. As previously described, specimens with signal ratios ≤2.0 were designated as non-amplified, while specimens with signal ratios >2.0 were designated as amplified (14).

Statistical Analysis

All statistical analyses were performed using Statistical Package for Social Sciences software version 19.0 software (IBM Corp.,
Armonk, NY, USA). Differences in ErbB2 gene and protein expression between osteochondroma and non-neoplastic tissue were analyzed using the Chi-square test or Fishers exact test, as were the relationships of those differences with clinical pathological data. Interobserver agreement in means of IHC staining and FISH results between two independent pathologists was analyzed using the kappa consistency test. p<0.05 was considered statistically significant.

Results

Clinicopathologic data of osteochondroma patients
The relevant clinicopathologic data of osteochondroma patients are summarized in Table 1. The study cohort included 20 males and 10 females with a mean age of 21.28±13.99 years (range, 5–61 years) at diagnosis, with 16 patients <18 years old and 14 patients ≥ 18 years old. A total of 21 patients presented with a tumor size <5 cm, while 9 patients presented with a tumor size ≥5 cm. A total of 27 patients presented with solitary osteochondroma, while 3 patients presented with multiple lesions. The most common site was located on humerus (8 patients), tibia (8), followed by femur (7), fibula (3), scapula (2), metacarpus (2), ilium (2), and phalanx (2), and finally radius in 1 patient. With regard to histologic features, 27 (90%) patients showed permeation of trabecular bone, 19 (63.33%) showed presence of binucleated cells, 18 (60%) showed presence of calcification, 17 (56.67%) showed presence of necrosis, 15 (50%) showed presence of nuclear pleomorphism and cystic/

Table 1. Clinicopathologic data for 30 osteochondroma patients

| Case | Age/Sex | Tumor size (cm³) | Subtype   | Tumor site           | Recurrence           |
|------|---------|------------------|-----------|----------------------|----------------------|
| 1    | 10/M    | 2.5*2.1*1.2      | Solitary  | Tibia                | No                   |
| 2    | 45/F    | 2.8*2.5*0.7      | Solitary  | Metacarpus           | No                   |
| 3    | 27/M    | 8.6*6.0*2.5      | Solitary  | Tibia                | Yes (24 months*)     |
| 4    | 5/M     | 5.5*4*1.5        | Multiple  | Tibia, humerus, femu| No                   |
| 5    | 8/M     | 4.5*4.2*2.5      | Solitary  | Tibia                | No                   |
| 6    | 8/M     | 3.5*1.5*1.2      | Solitary  | Femur                | No                   |
| 7    | 11/F    | 6*4*3            | Solitary  | Fibula               | No                   |
| 8    | 10/M    | 2.5*1.7*1.3      | Solitary  | Humerus              | No                   |
| 9    | 12/F    | 4.3*2.3*1.7      | Solitary  | Humerus              | No                   |
| 10   | 12/M    | 1.5*1.2*1.1      | Solitary  | Scapular             | No                   |
| 11   | 38/F    | 2.5*2*1.2        | Solitary  | Humerus              | No                   |
| 12   | 10/M    | 3*2.7*1          | Solitary  | Femur                | Yes (9 months*)      |
| 13   | 10/M    | 4.8*1.7*1        | Solitary  | Fibula               | No                   |
| 14   | 17/M    | 2*1*1            | Solitary  | Radius               | No                   |
| 15   | 14/M    | 10*6*2           | Multiple  | Tibia, humerus, femu| No                   |
| 16   | 33/M    | 7*7*3.8          | Solitary  | Humerus              | No                   |
| 17   | 13/M    | 6*4*3            | Solitary  | Fibula               | No                   |
| 18   | 34/M    | 5.5*5*4.5        | Solitary  | Scapular             | No                   |
| 19   | 27/M    | 6*5.5*2          | Solitary  | Tibia                | No                   |
| 20   | 24/F    | 3.5*1*1.5        | Multiple  | Tibia, humerus       | No                   |
| 21   | 52/F    | 1.0*0.5*1.2      | Solitary  | Metacarpus           | No                   |
| 22   | 19/M    | 3*3*3            | Solitary  | Femur                | No                   |
| 23   | 10/M    | 4.5*2.5*1.5      | Solitary  | Femur                | No                   |
| 24   | 14/F    | 3.5*1.5*0.8      | Solitary  | Humerus              | No                   |
| 25   | 61/M    | 2.6*1.9*1.8      | Solitary  | Phalanx              | No                   |
| 26   | 23/F    | 2.5*1.8*1.4      | Solitary  | Femur                | No                   |
| 27   | 20/M    | 4.4*4.3*3.5      | Solitary  | Ilium                | No                   |
| 28   | 22/M    | 5.5*4.5*3.6      | Solitary  | Ilium                | No                   |
| 29   | 16/F    | 1.8*1.2*0.6      | Solitary  | Phalanx              | No                   |
| 30   | 19/F    | 1.6*2.0*1.1      | Solitary  | Tibia                | No                   |

*After surgery; M: male; F: female
mucoid changes, 13 (43.33%) showed presence of nodularity, 12 (40%) showed high expression of cellularity, and 5 showed presence of mitosis. Radiographically, 22 cases demonstrated sessile osteochondroma, while 8 showed pedunculated osteochondroma. A total of 4 patients showed cartilage thickness ≥2 cm, while 3 showed presence of cap volume ≥2 cm.

The mean follow-up time was 23.43±4.56 months (range: 15–32 months). Two osteochondroma patients locally relapsed by the follow-up date.

ErbB2 gene and protein expression in osteochondroma and non-neoplastic bone tissue

Of the 30 osteochondroma specimens, 4 cases (13.3%) presented with ErbB2 protein over-expression, which was only seen in 1 of 20 (5%) non-neoplastic bone tissues (Figure 1, Table 2), based on a good interobserver agreement between the two independent pathologists (kappa coefficient=0.786, p<0.001). No significant difference of ErbB2 protein over-expression between the two groups was observed (p=0.336, Table 2). FISH results from the 30 osteochondroma specimens demonstrated a mean signal ratio of 2.44±1.66, with 13 cases (43.3%) displaying amplification of the ErbB2 gene (Figure 2, Table 2). Figure 3 shows FISH signals in nuclei from different regions (perichondrium, cartilage cap, osteocytes, and osteoblasts) in one H&E-stained section of osteochondroma. A relatively low mean signal ratio of 1.16±0.10 was observed for the 20 non-neoplastic bone tissues, with no cases displaying gene amplification (Figure 2, Table 2). The FISH results also achieved good interobserver agreement between the two independent pathologists (kappa coefficient=0.779, p<0.001). The difference in ErbB2 gene amplification between the two groups was found to be statistically significant (p<0.001, Table 2). Two cases with osteochondroma showed both ErbB2 gene amplification and protein overexpression, while 15 cases showed negative results for both. On the other hand, 11 gene-amplified cases showed low protein expression, and 2 gene-non-amplified cases showed protein overexpression. Significant concordance between FISH and IHC results was not observed (kappa coefficient=0.039, p=0.773).

Association between ErbB2 expression and clinicopathological features of osteochondroma

The relationship between clinicopathological features of osteochondroma and ErbB2 gene and protein status is summarized in Table 3. The amplification of the ErbB2 gene was closely associated with adverse histological parameters of osteochondroma, including high expression of cellularity (p<0.001), presence of binucleated cells (p<0.001), nuclear pleomorphism (p=0.003), calcification (p=0.002), nodularity (p=0.002), and necrosis (p=0.009), as well as cartilage thickness ≥2 cm as a radiographical feature (p=0.026). No significant association of gene amplification with other histological parameters, including permeation of trabecular bone (p=0.238), cystic/mucoid changes (p=0.139), and mitosis (p=0.628), was observed. In terms of radiographic features, differences in ErbB2 gene expression were not observed for differences in appearance (sessile and pedunculated osteochondromas) in cap volume, or in osteochondroma subtype. Meanwhile, the over-expression of ErbB2 protein was not con-

**Table 2. ErbB2 status between osteochondroma and corresponding non-neoplastic bone tissue**

| ErbB2 status  | Osteochondroma (%) | Non-neoplastic tissue (%) | p     |
|---------------|---------------------|---------------------------|-------|
| Protein   |                     |                           |       |
| High expression | 4 (13.33)          | 1 (5)                     | 0.336 |
| Low expression | 26 (86.67)         | 19 (95)                   |       |
| Gene       |                     |                           |       |
| Amplification | 13 (43.33)         | 0 (0)                     | 0.001 |
| Non-amplification | 17 (56.67)       | 20 (100)                  |       |

**Figure 1. a, b. Expression of the ErbB2 protein in osteochondroma and non-neoplastic bone tissue. Over-expression of the ErbB2 protein in osteochondroma (a). Low expression of the ErbB2 protein in non-neoplastic bone tissue (b)**
Figure 2. a, b. Expression of the ErbB2 gene in osteochondroma and non-neoplastic bone tissue. ErbB2 gene amplification in osteochondroma (a). ErbB2 gene non-amplification in non-neoplastic bone tissue (b). Red signal, fluorophore-labeled DNA of the ErbB2 gene locus; green signal, fluorophore-labeled alpha satellite DNA of chromosome 17.

Figure 3. A hematoxylin-eosin-stained section of osteochondroma (left panel) and FISH results of interphase nuclei from indicated osteochondroma regions (right panels). Red signal, fluorophore-labeled DNA of the ErbB2 gene locus; green signal, fluorophore-labeled alpha satellite DNA of chromosome 17.
nected with the clinicopathological parameters of osteochondroma stated above.

**Discussion**

Although osteochondroma is currently defined as a benign disease (15), the pathogenesis of osteochondroma remains unclear (16). Despite usually presenting as asymptomatic neoplasm (17), osteochondroma in a small subset of patients may locally recur or transform malignantly to chondrosarcoma. The present study investigated the role of ErbB2 gene and protein expression on osteochondroma, with results showing that the amplification of the ErbB2 gene was associated with an unfavorable clinicopathological state of osteochondroma. This finding may serve as a valuable molecular marker for the malignant tendency of osteochondroma.

The ErbB2 gene is an oncogene originally found in the DNA of rat neuroblastoma and glioblastoma in the 1980s (18). The role of the ErbB2 gene in breast cancer and gastric cancer has been clearly defined. A previous study by Radenkovic et al. demonstrated that ErbB2-positive breast cancer represented a highly aggressive breast cancer subtype, and that stronger ErbB2 staining was more frequent in cancer with a worse prognosis (19). Amplification of the ErbB2 gene enhances telomerase activity, which promotes the cell cycle of tumor cells, tumor angiogenesis, and invasion, while inhibiting tumor cell apoptosis (20). Additionally, the ErbB2 gene could serve as a therapy target to improve survival rate (21).

### Table 3. Association of ErbB2 status with clinicopathological features of osteochondroma

| Clinicopathological features (n, %) | IHC | FISH | p | Non-amplification | p |
|-----------------------------------|-----|------|---|-------------------|---|
|                                   | Over-expression | Low-expression |       | Amplification     | Non-amplification |       |
| Histologic parameters             |     |      |   |                   |               |     |
| Cellularity                        |     |      |   |                   |               |     |
| High (12, 40)                      | 2   | 10   | 1.000 | 10               | 2               | 0.001 |
| Low (18, 60)                       | 2   | 16   | 0.268 | 13               | 6               | <0.001 |
| Binucleated cells                  |     |      |   |                   |               |     |
| Present (19, 63.33)                | 4   | 15   | 0.100 | 11               | 4               | 0.003 |
| Absent (11, 36.67)                 | 0   | 11   | 0    | 0                 | 11              |       |
| Nuclear pleomorphism               |     |      |   |                   |               |     |
| Present (15, 50)                   | 4   | 11   | 0.100 | 11               | 4               | 0.003 |
| Absent (15, 50)                    | 0   | 15   | 0    | 0                 | 11              |       |
| Calcification                      |     |      |   |                   |               |     |
| Present (18, 60)                   | 2   | 16   | 1.000 | 12               | 6               | 0.002 |
| Absent (12, 40)                    | 2   | 10   | 1    | 1                 | 11              |       |
| Nodularity                         |     |      |   |                   |               |     |
| Present (13, 43.33)                | 2   | 11   | 1.000 | 10               | 3               | 0.002 |
| Absent (17, 56.67)                 | 2   | 15   | 3    | 14                |               |       |
| Permeation of trabecular bone      |     |      |   |                   |               |     |
| Present (27, 90)                   | 3   | 24   | 0.360 | 13               | 14              | 0.238 |
| Absent (3, 10)                     | 1   | 2    | 0    | 3                 |               |       |
| Cystic/mucoid changes              |     |      |   |                   |               |     |
| Present (15, 50)                   | 1   | 14   | 0.598 | 9                | 6               | 0.139 |
| Absent (15, 50)                    | 3   | 12   | 4    | 11                |               |       |
| Necrosis                           |     |      |   |                   |               |     |
| Present (17, 56.67)                | 1   | 16   | 0.290 | 11               | 6               | 0.009 |
| Absent (13, 43.33)                 | 3   | 10   | 2    | 12                |               |       |
| Mitosis                            |     |      |   |                   |               |     |
| Present (5, 16.67)                 | 1   | 4    | 0.538 | 3                | 2               | 0.628 |
| Absent (25, 83.33)                 | 3   | 22   | 10   | 15                |               |       |
| Radiographic features              |     |      |   |                   |               |     |
| Appearance                         |     |      |   |                   |               |     |
| Sessile (22, 73.33)                | 2   | 20   | 0.284 | 8                | 14              | 0.242 |
| Pedunculated (8, 26.67)            | 2   | 6    | 5    | 3                 |               |       |
| Cartilage thickness                |     |      |   |                   |               |     |
| ≥2 cm (4, 13.3)                    | 1   | 3    | 1.000 | 4                | 0               | 0.026 |
| <2 cm (26, 86.7)                   | 3   | 23   | 9    | 17                |               |       |
| Cap volume                         |     |      |   |                   |               |     |
| ≥2 cm³ (3, 10)                     | 0   | 3    | 1.000 | 3                | 0               | 0.100 |
| <2 cm³ (27, 90)                    | 4   | 23   | 10   | 17                |               |       |
| Type                               |     |      |   |                   |               |     |
| Solitary (27, 90)                  | 4   | 23   | 1.000 | 13               | 14              | 0.238 |
| Multiple (3, 10)                   | 0   | 3    | 0    | 3                 |               |       |
differentiate osteochondromas from malignant cartilaginous tumors included degree of cellularity, binucleated cells, nuclear pleomorphism, calcification, nodularity, permeation of trabecular bone, cystic/mucoid changes, necrosis, and mitosis (22). In the present study, we found that the amplification of the ErbB2 gene was significantly related with high expression of cellularity, binucleated cells, nuclear pleomorphism, calcification, nodularity, and necrosis of osteochondroma, which had not been reported in previous studies.

High cellularity has been identified as a significant parameter for differentiating enchondroma from low-grade chondrosarcoma (22). Additionally, one recent study noted signaling related to chondrosarcomatous transformation, the elevation of which increased cellularity and inhibited chondrocyte differentiation in osteochondroma, leading to the predisposed conversion into chondrosarcoma (23). ErbB2 signaling in the progression mechanisms of osteochondroma is worthy of further investigation. Nuclear pleomorphism has been confirmed as a hallmark of high-grade secondary chondrosarcoma by a previous study (24). Changes in chondroid calcification, even soft-tissue swelling containing calcification, if observed, are very suggestive of malignant progression into a secondary chondrosarcoma (24). Nodules are often observed in low-grade chondrosarcomas and in active cartilaginous lesions. The presence of nodularity in osteochondromas was considered as a feature of likely malignant transformation giving rise to surgical resection (22).

According to the authors of a previous study, however, histologic parameters of chondrosarcoma are subjected to substantial interobserver variability, so that a multidisciplinary assessment combining histologic parameters with radiographical features may improve the reliability of cartilaginous tumor diagnosis (22). Radiographically, cartilage thickness is a notable indicator for evaluating the malignant potential of an osteochondroma, with values greater than or equal to 2 cm strongly considered as a sign of development from osteochondroma to secondary chondrosarcoma (25). A subsequent study confirmed this means of distinguishing osteochondroma from secondary chondrosarcoma by cartilage thickness (24). In the present study, a statistically significant association between ErbB2 gene amplification and cartilage thickness in osteochondroma was found, indicating the predictive role of ErbB2 in chondrosarcomatous conversion of osteochondroma.

Generally, the ErbB2 protein is over-expressed as a result of gene amplification (26). Notably, our study found an ErbB2 gene amplification rate (43.3%) higher than the ErbB2 protein over-expression rate (13.3%). At the same time, significant association of ErbB2 protein over-expression with clinicopathological parameters of osteochondroma was not observed. A comprehensive, blind pathology evaluation by Perez et al. showed discordant results between IHC and FISH assessment of ErbB2 expression in breast cancers, with the disagreement likely attributed to ErbB2 tumor heterogeneity (27). Paik et al. conducted initial trials enrolling metastatic breast cancer patients with positive staining of ErbB2 by IHC for ErbB2-targeted therapy, only to find in subsequent studies that the benefit was limited to tumors with ErbB2 amplification as determined by FISH (28). Another study by Mattsson et al. identified poor concordance in the detection of anaplastic lymphoma kinase (ALK) rearrangements between IHC and FISH assays, leading them to question current strategies screening with IHC prior to FISH or completely replacing FISH with IHC (29). A comprehensive approach integrating IHC and FISH assays is therefore optimal for accurate and consistent evaluation of ErbB2 status (14).

The present study presents certain limitations. First, as a retrospective study for which some information was missing for some patients, this study may be biased in its collection of patients’ data. Second, the number of cases included in our study is relatively small. A future prospective study with larger sample size and more follow-up data is required to confirm the present study’s results.

In summary, the present study observed ErbB2 gene amplification and ErbB2 protein overexpression in osteochondroma. In addition, ErbB2 gene amplification was found to be associated with histologic parameters including high expression of cellularity, presence of binucleated cells, nuclear pleomorphism, calcification, nodularity, and necrosis, as well as with cartilage thickness as a radiographical feature. The ErbB2 gene could serve as a predictor in evaluating the potential for malignant conversion of osteochondroma into secondary chondrosarcoma. Further research is required to confirm the predictive value of ErbB2 identified in the present study for osteochondroma.

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