RESEARCH ARTICLE

Discovery and Characterization of Intercondylar Transphyseal Complexes and their Oncological Significance in Transphyseal Extension of Pediatric Osteosarcoma

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Objective: To explore whether there exist undiscovered transphyseal vasculature-canal compound structures in immature femurs and tibias, and reveal their potential oncological impact.

Methods: This investigation was divided into a morphological study and a clinical study. In the morphological part, a new-identified anatomic structure was investigated by using radiographical, anatomical, and histological methodologies. Twenty-eight 1-mm-slice thickness magnetic resonance images of pediatric knees were generated and 10 pediatric knees were dissected to verify the existence and universality, observe the radiographic and anatomic characteristics, and determined the located region of this structure. Hematoxylin–eosin staining, immunofluorescence, and angiography procedures were performed to illustrate its histological feature, molecular identification, and vascular origination, respectively. In the clinical part, 38 pediatric osteosarcoma patients were enrolled from January 2014 to December 2020. A descriptive clinical study including 13 typical participants was conducted to investigate the oncological significance of this new-identified structure. Meanwhile, the discrepancy in transphyseal osteosarcoma extension between different physeal regions was evaluated in a cross-sectional study.

Results: In the morphological study, we discovered a new-found vasculature-canal compound structure, intercondylar transphyseal complex (ITC), which originated from the middle genicular vessels, traversed the whole epiphysis, and breached the intact open physis in the immature proximal tibia or distal femur. The components of ITC included the juxta-articular, epiphyseal, and transphyseal segments of vessels, the canals that traverse the entire epiphysis and physis and enclosed the vessels, vascular foramina on articular facet and foramina-covered synovium. Depending on the location, ITCs can be divided into three types: femoral ITC, anterior tibial ITC, and posterior tibial ITC. Clinically, the ITC may facilitate intercondylar transphyseal sarcomatous dissemination without damaging the adjacent physeal cartilage. Compared to bilateral condylar physes, more osteosarcomas transgressed the open growth plates through intercondylar regions in which ITC was located ($P = 0.022$).

Conclusion: As the "gap" on intact open physis, ITC, which is a new-identified compound structure in intercondylar regions of immature femur or tibia, may promote intercondylar transphyseal tumor extension. Moreover, the

identification and characterization of ITC subvert some traditional comprehensions about physis and may provide novel perspectives for pediatric osteosarcomas.

Key words: Growth plate; Intercondylar transphyseal complex; Osteosarcoma; Transphyseal tumor extension; Vasculature-canal structure

Introduction

Transphyseal tumor extension is an important phenomenon that may affect the treatment strategies and comprehensive prognoses in pediatric oncology[1,2]. Therefore, the tumor-generated vasculatures and canals which facilitate the tumor transphyseal dissemination have been extensively studied in current research[3,4]. However, the extension of neoplasm *via* natural transphyseal vasculature-canal structure has not been systemically investigated. In our recent investigation of mature tibias and femurs, we discovered a new-found vasculature-canal structure called Lijianmin-Chengkun complex that originates from the genicular vessel networks and deeply penetrates into the tibia or femur[5]. This discovery not only contributed to local recurrence and bidirectional dissemination of adult knee-related tumors but also provided a novel conjecture for potential undiscovered vasculature-canal complex structures and their relevant oncological significance in immature tibia and femur, especially for the potential clinical significance of how these structures mediate the transphyseal tumor extension.

Based on current research, it is believed that the growth plate in children after reaching 2 years old is an intact structure, with the cells tightly arranged, through which there are no voids or canals[6]. In traditional views, transphyseal vessels which arise from periosteal vessels only exist in the neonatal and infant periods[7]. However, there is no report about the vessels and canals that traverse the entire epiphysis and physis in children of 4–15 years with open growth plates[8]. Moreover, the genicular vessel system, which mainly originates from the popliteal vasculature and has been subjected to considerable research in adults[9], has rarely been discussed in reports that investigated the physeal, epiphyseal, and metaphyseal vessels in children.

Osteosarcoma (OS), the most common primary sarcoma affecting the lower extremities in skeletally immature individuals, has attracted considerable attention in various studies in recent decades[10–12]. Currently, open physes are often regarded as a natural barrier to osteosarcoma invasion because of their high avascularity, continuity, and cell density, and few people expect them to have a "gap"[3,13]. However, a series of interesting phenomena were observed in our clinical works: it seems that more metaphyseal osteosarcomas tend to penetrate the growth plates through intercondylar regions rather than bilateral condyles in proximal tibias and distal femurs. For patients with intercondylar transgression, the breached site will sometimes be a "breakpoint" on the growth plate with peripheral uninvaded cartilage; however, this feature was seldom found in bilateral condylar physes. These observations inspired us to propose two hypotheses: first, is the resistance to OS different between the intercondylar and bilateral condylar growth plates? Second, does there exist any undiscovered "dangerous channels" for tumor dissemination in the intercondylar region of the immature femur and tibia? To evaluate the tumorous region and choose the surgical interventions, a general classification of the location and extent of physeal and epiphyseal sarcomatous lesions based on magnetic resonance imaging (MRI) has already been established[2]; however, as existing problems, there were few quantitative comparisons of the difference between intercondylar and bilateral condylar transphyseal osteosarcoma extension in previous investigations, nor have the risk factors been investigated that cause the potential discrepancy of resistance to osteosarcoma between these regions.

OS is a well-vascularized tumor making antiangiogenic therapy a hot topic in clinical strategies[14,15]. The growth plate has generally been considered a barrier to the penetration by osteosarcoma; therefore, its predominantly avascular feature and tight physeal chondrocyte arrangements play important roles[3,7]. It is also believed that scarcely any canals or vessels exist through which the tumor transphyseal extension on normal growth plates may be facilitated[16]. Microscopically, the main barrier function of the growth plate to OS is based on the resting, proliferative, and upper hypertrophic chondrocyte cells with high germinal ability and abundant extracellular matrix[4,7]; in addition, these layers are mainly avascular with high expression of antiangiogenic pigment epithelium-derived factor[4]. Moreover, it is also believed that tumorous angiogenesis plays a vital role in OS spread, including transphyseal extension, because these vessels may invade and damage normal tissues, thereby subsequently acquiring intravasation and spreading to further areas[4]. However, there is an existing problem that the explorations of transphyseal tumor extension through natural transphyseal canals and vessels are extremely limited.

Based on the background and our previous study[5], we discovered a new-found structure called intercondylar transphyseal complex (ITC) in children, which may be a vital structure to facilitate the transphyseal tumorous extension. This study aims to systemically investigate the anatomical and clinical significance of ITC as follows: (i) to determine the normal morphological characteristics of ITCs, including the properties of each component of ITC, the occurrence rate of ITC in normal children, its relative location in immature distal femur and proximal tibia, the histological characteristics of each component of ITC, and the molecular

identification of its enclosed vasculature-like structures; (ii) to preliminarily analyze the radiographical features and pathological features of ITCs in pediatric OS patients; (iii) to investigate the clinical significances of ITCs by evaluating whether ITC may facilitate the transphyseal OS extension in pediatric femur and tibia.

Material and Methods

All studies were conducted in Qilu Hospital, Qilu Hospital (Qingdao), and the Department of Anatomy and Neurobiology of Shandong University with the approval of the institutional ethics committee (No. LL-2016-1-040). All the participants' guardians in clinical studies signed relevant informed consent forms. For the detailed information of the patients, please see the Supporting Information.

Morphological Study

Material Selection

From March 2017 to June 2020, 28 MRIs and 10 dissections of pediatric knees were enrolled in the morphological study, with the inclusion and exclusion criteria as follows.

The inclusion criteria were: (i) complete and clear MRIs of the knee joint with the consecutive sequences; (ii) intact knee joint specimens obtained from cadavers or amputated extremities; (iii) age from 4–15 years with intact open growth plates; (iv) radiographic observations and measurements were conducted on the MRIs, and the dissections were utilized for anatomical study, hematoxylin–eosin (HE) staining, and immunofluorescence (IF); (v) outcomes, including the radiographic and anatomic observations of each component of ITC, the radiographic and anatomic measurements for the location of ITC, the histological characterizations of each component of ITC, and the molecular identification of ITC, were provided; (vi) a descriptive study.

The exclusion criteria were: (i) the senescent or closed epiphyseal lines under eligible age; (ii) the growth plate or articular facet with serious damage or maldevelopment in either distal femur or proximal tibia; (iii) important structures in femoral intercondylar notch or tibial intercondylar region with severe injury; (iv) the malformation or severe destruction in middle genicular artery (MGA) or middle genicular vein (MGV); (v) the MRIs or dissections with improper reservation; (vi) history of surgery or invasive examination of knee.

Radiographic Observation and Measurement of ITC

The ITCs were identified and quantitatively examined in 28 MRIs, their relative locations on the growth plate and articular surface in the sagittal direction were measured with the stipulated parameters (Table 1). All MRIs were performed on two 3-T MR scanners (Magnetom Verio3.0T) with dedicated 8-channel knee coils, slice thickness of 1 mm, and no gap. An imaging illustration of the measured parameters is displayed (Figure 1). Whether the ITCs were located in intercondylar or bilateral condylar regions was also

TABLE 1 Definition of the measured parameters indicating the location of tibial ITC (mm)

Region	Definition	Explanation
Tibial ITC		
Articular surface	DAA	The distance between the vascular foramen of ITC and anterior margin of tibia plateau
	DPA	The distance between the vascular foramen of ITC and the intersection point of DAA extending line and the sagittal line on which the posterior margin of tibia lay
Growth plate	DAP	The distance between transphyseal site of ITC and anterior terminal point of the "physeal line" in tibia
	DPP	The distance between transphyseal site of ITC and posterior terminal point of the "physeal line" in tibia
Femoral ITC		
Articular surface	DAA	The distance between vascular foramen of ITC and anterior margin of ICF
	DPA	The distance between vascular foramen of ITC and posterior margin of ICF
Growth plate	DAP	The distance between transphyseal site of ITC and anterior terminal point of the "physeal line" in femur
	DPP	The distance between transphyseal site of ITC and posterior terminal point of the "physeal line" in femur

Note: The "physeal line" on which DAP and DPP lie is parallel to the proximal tibial or distal femoral growth plate for the most part. In tibia, the anterior terminal point of "physeal line" is often created by extending the posterior part of physes to the anterior margin of tibia to diminish the potential error caused by the development of tibial tubercle. All the "margins" are situated in the corresponding MRI layer which the relevant structures appeared.; Abbreviation: ICF, intercondylar fossa.
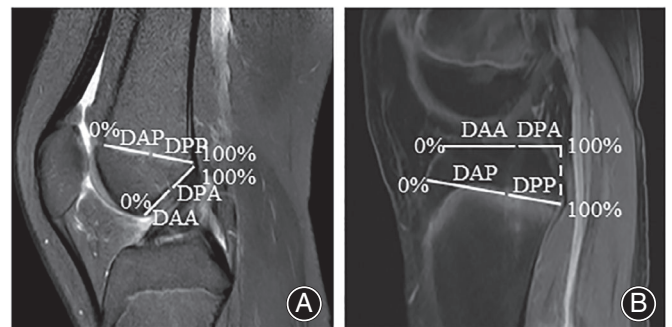
Fig. 1 Illustration of the parameters for indicating the location of femoral and tibial ITCs. (A) The measurement of DAA, DPA, DAP, and DPP in femoral ITC on sagittal MRI. (B) The measurement of DAA, DPA, DAP, and DPP in tibial ITC on sagittal MRI. To evaluate the relative location of ITC on the articular facet and physis, the ratio of DAA/(DAA + DPA) and DAP/(DAP+DPP) were expressed as a percentage from 0% to 100%.

recorded. To ensure the authenticity of ITC, all observations and measurements were performed in three planes (axial, coronal, sagittal) and confirmed by at least two researchers.

Anatomic Dissection of ITC

To characterize the ITC in gross anatomy, 10 healthy pediatric knees from four cadavers and two amputated lower extremities underwent the dissection. The juxta-articular segments were dissected as previously described[5]. Meanwhile, we also dissected the transphyseal segments of ITCs to observe their anatomic features. Using the same parameters as MRI in the sagittal direction, the locations of ITCs on the articular surface and growth plate were measured during dissection. We also documented the number of ITCs in the intercondylar region and whether such structures appeared in the bilateral condylar region. After dissection, each component of ITC was sent for histological examination via HE staining. IF was performed to identify the molecular characteristics of ITC vessels.

Angiography of ITC

To verify the location of the tibial or femoral ITC vessels and whether these vessels breach the growth plates, we visually examined the vasculature after injecting the contrast agent (300 mg/ml iohexol) into the popliteal vessels of the specimens. The imaging of intraosseous ITC vessels and their originations in the knee joint were recorded using a video camera.

HE Staining and IF

HE staining was performed to analyze the histological features of ITCs, as previously described[17]. The juxta-articular and transphyseal vessels in the normal ITCs were sent for double-labeled IF examination to verify the identity of the vasculatures. The differentiation between arteries and veins depends on the preferential expression of ephrin-B2 and Eph-B4, respectively[18]. The routine steps of IF were performed as previously described.[19] Primary antibodies in double-labeled IF were Eph-B4 and ephrin-B2, Goat Anti-Mouse CY3 and goat anti-rabbit 488 were chosen as secondary antibodies, and DAPI was used for counterstaining. The tissue section mounted slides were examined under an Olympus IX-70 microscope (Tokyo, Japan) and a NIKON ECLIPSE C1 microscope (Nikon, Japan).

Clinical Study

Patient Selection

From January 2014 to December 2020, a patient cohort including 38 pediatric patients (21 males and 17 females, an average age of 11.53 ± 2.79 years [6–15 years]) with pathologically confirmed OS in the proximal tibia or distal femur was established. With this cohort we analyzed the clinical oncological significances of femoral and tibial ITCs in pediatric osteosarcomas. The inclusion and exclusion criteria are illustrated below.

Inclusion Criteria. (i) Patients under 16 years of age with open physes; (ii) patients who underwent MRI for the lesion regions with adequate consecutive sequences; (iii) patients with pathologically confirmed OS that invaded or surpassed the growth plate in the distal femur or proximal tibia; (iv) the different transphyseal regions of osteosarcoma were compared; (v) outcomes included the radiographical and pathological observations, radiographical assessment, the record of different lesion types, and the discrepancy between different transphyseal regions; (vi) a descriptive study and a cross-sectional study.

Exclusion Criteria. (i) A partly or totally closed epiphyseal line in distal femur or proximal tibia; (ii) an indistinct boundary between normal cartilaginous physes and adjacent epiphysis; (iii) uncertain radiographic characteristics to distinguish between the sarcomatous region and edematous or hematopoietic lesions; (iv) a tumor-invaded region of the growth plate denied by postoperative pathological examination; (v) an unrecognizable articular facet.

Clinical Descriptive Study

Typical tumor involvements in ITC were elaborated in 13 osteosarcoma patients (eight boys and five girls), with an average age of 12.08 ± 1.89 years (9–15 years old) who underwent segmental resection or amputation in the descriptive clinical study. Tumor involvement in their ITCs was verified using radiographic, intraoperative, and pathological methodologies. With the agreement of the patients' guardians, the 13 knees were dissected and HE staining was performed to analyze the characteristics of sarcomatous invasion in the ITC.

Clinical Cross-Sectional Study

To study whether the ITCs facilitate transphyseal OS extension, we divided the growth plate into intercondylar and bilateral condylar regions. The boundaries between intercondylar and medial/lateral condylar regions in the proximal tibia and distal femur were formulated based on previously described.[5,20] The extent of tumorous extension was classified referring to the epiphyseal cartilage and the joint surface into four types (Table 2).

The discrepancy in oncological resistance between intercondylar and bilateral condylar physes was judged by whether more osteosarcomas breached the growth plate in one region than the other when they touched the physes, based on the quantitative difference between Type I lesions and Type II–III lesions. All the tumor-involved areas were first determined by MRI[21], and then confirmed by intraoperative observation and postoperative histopathological examination. Radiographic features of how ITC mediated the transphyseal extension were also revealed.

TABLE 2 Classification and explanation of lesion types		
Lesion type	Lesion characteristic	Explanation
Type 0	Lesion without physeal involvement	The tumor does not reach the physes
Type I	Physes-involved lesion	The tumor touches the physes without transgression
Type II	Transphyseal but not transepiphyseal lesion	The tumor breaches the growth plate and is located within epiphysis
Type III	Transepiphyseal lesion	The tumor extends to or beyond the articular cartilage

Statistical Analysis

Student's *t*-test was performed to analyze significant differences in the location information of normal ITC between MRI and anatomic specimens. A chi-square test was performed to statistically compare the number of patients with Type I lesions and Type II–III lesions through different physeal regions. The Kolmogorov–Smirnov (K-S) test was used to calculate the distribution range of femoral and tibial ITC locations on articular facets and physes. SPSS 21.0 and MATLAB 2016b were used for all the statistical calculations. $P < 0.05$ was set for data to be statistically significant.

Results

The Identification and Characterization of ITC in Morphological Study

With the utilization of radiographic, anatomical, histological, and molecular methodologies, we discovered a new vasculature-canal compound structure, ITC, which consists of juxta-articular, epiphyseal, and transphyseal segments of vessels, canals that traverse the entire epiphysis and physis, vascular foramina on the articular facet, and foramina-covered synovium in the intercondylar region of the distal femur and proximal tibia.

Using 1-mm-thick MRIs ($n = 28$), complete ITCs were observed in 27 femurs (96.4%) and 28 tibias (100%). Some femoral ITC vessels coalesced centrally into one vessel in the epiphysis (Figure 2A); however, such coalescence was never observed in the tibia (Figure 2B,C). Furthermore, several ITCs penetrated into the tibial epiphysis very close to the anterior margin of the tibia plateau while other ITCs penetrated into the central-to-posterior region (Figure 2B,C). In each knee on MRI, 2–4 and 1–2 foramina on the articular surface were discovered in the femoral and tibial ITCs, respectively. Meanwhile one or two transphyseal sites of femoral and tibial ITCs were shown (Figure 2D). In contrast to the location of the femoral ITC which obeys the normal distribution, two peaks were found in the distribution of tibial ITCs on the joint facet (Figure 2E). Based on statistical analysis, we divided the tibial ITCs into anterior and posterior

tibial ITCs according to their sagittal boundary value of 46.35% on articular facet (Table 3).

ITCs were found in all 10 dissected pediatric knees. The vascular foramina of the ITC on the tibial and femoral articular facet are covered by soft tissues (Figure 2F). All ITC vessels penetrated the surrounding articular cartilage, traversed the entire epiphysis and physis, and reached the metaphysis within the bony canals (Figure 2G,H), and these vessels originated from the branches of MGA or the tributaries of MGV (Figure 2F–H). We also counted the number and determined the location of ITCs using dissections (Table 4). The number and location of juxta-articular vascular foramina or transphyseal sites in anatomical dissections were highly consistent with that in radiographic observations, showing no statistically significant differences of femoral ITCs, anterior tibial ITCs, and posterior tibial ITCs ($P = 0.525$, 0.470 and 0.134 on articular facet, respectively; $P = 0.417$, 0.200 and 0.971 on physes, respectively) (Figure 2I,J). Besides, both femoral and tibial ITCs were clearly visible utilizing angiographic techniques (Figure 2K). All ITCs were shown in intercondylar region in radiographic and anatomic observations.

The histological and molecular characteristics of the components of ITC were revealed by HE staining and IF. The results showed that the foramina-covered soft tissues consisted of typical synovial cells and loose connective tissues (Figure 3A). The vasculatures in ITCs are small veins or arteries (Figure 3B). The histologic features of the walls of epiphyseal ITC canals are similar to those of adjacent epiphysis (Figure 3C). These canals also transgress the intact growth plate, causing the adjacent physeal cartilage to be completely interrupted, and no physeal chondrocytes were detected in the transphyseal canals of ITCs (Figure 3D). The juxta-articular and transphyseal segments of ITC vessels were sent for IF and HE staining to examine their vascular identity. Single positive expression of ephrin-B2 or Eph-B4 was shown in anterior or posterior tibial ITC vessels, respectively (Figure 3E,F). However, in a femoral ITC that may contain multiple juxta-articular and transphyseal vessels, either a single positive arterial or venous feature was shown in each vessel (Figure 3G,H). These findings were also supported by the vascular features of HE staining (Figure 3I-K).

### Osteosarcoma Extension via ITC in Clinical Descriptive Study

In the descriptive clinical study, 12 patients had osteosarcomas that breached the growth plate through ITC canals, whereas the peripheral physeal cartilage was relatively intact (Figure 4A,B). For the furthest extension, the tumor traversed the entire growth plate and epiphysis to the articular cavity (Figure 4C,D). Under the microscope, the pathological features showed that osteosarcoma cells perforated the growth plate through ITC canals without damaging adjacent physeal cartilage by HE staining (Figure 4E). One patient showed that the metaphyseal ITC vessels were involved in

OS, while the tumor margin did not reach the growth plate (Figure 4F).

Evaluation for Transphyseal Osteosarcoma Extension in Clinical Cross-Sectional Study

Based on a cross-sectional study of 38 physis-related osteosarcoma patients (Supporting Table S1), we investigated the discrepancy in transphyseal tumor extension between the intercondylar region where ITC located and bilateral condylar region. Type I–III lesions with tumorous involvement in the intercondylar and bilateral condylar physes were observed in 37 and 33 patients, respectively. Among them, 33 and 22 transphyseal OS extensions (including transepiphyseal lesions) occurred with intercondylar and bilateral
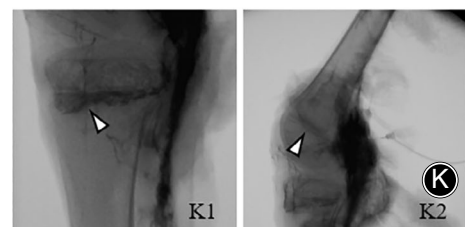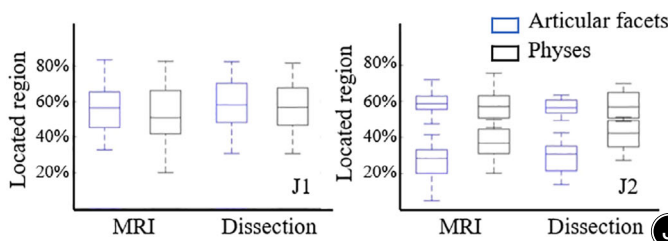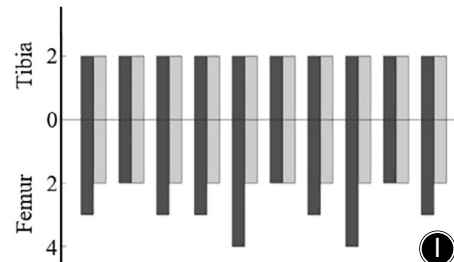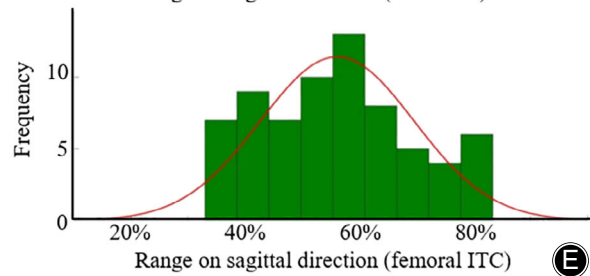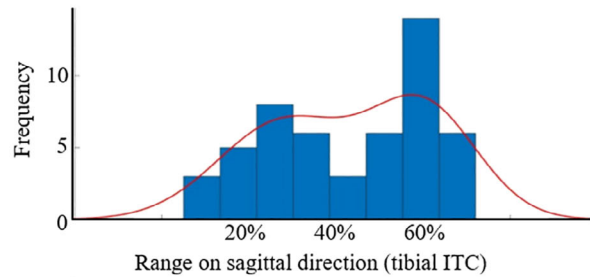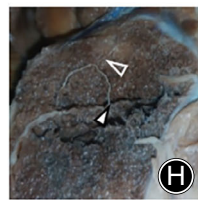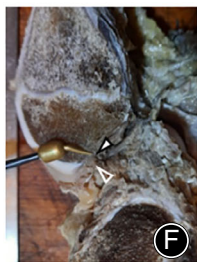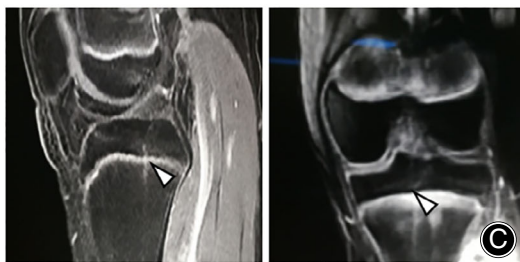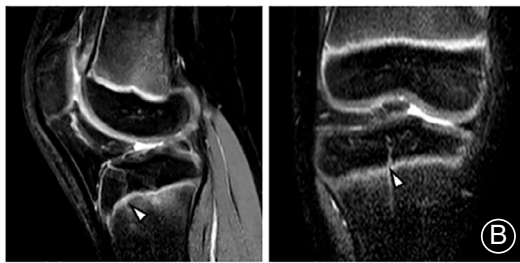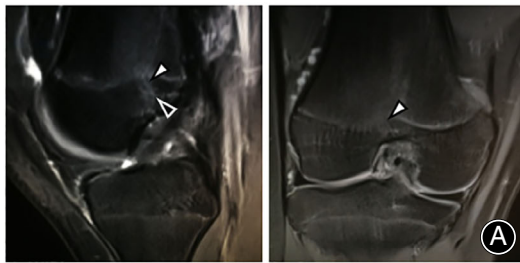
TABLE 3 Number and location of femoral and tibial ITCs in MRI(%)

	Number	Mean sagittal location on articular facet	Sagittal located region on articular facet	Mean sagittal location on physis	Sagittal located region on physis
Femoral ITC	27	56.13 ± 13.49	29.69–82.57	53.67 ± 15.05	24.17–83.19
Anterior tibial ITC	25	26.61 ± 9.48	8.03–45.19	36.91 ± 8.61	20.03–53.79
Posterior tibial ITC	26	59.55 ± 6.34	47.12–71.98	57.64 ± 8.03	41.90–73.38

Note: The mean sagittal locations on articular facet and physis were calculated by DAA/(DAA + DPA) and DAP/(DAP + DPP). The sagittal located regions were calculated by the 95% confidence interval (CI) with K-S test.

TABLE 4 Number and location of femoral and tibial ITCs in anatomic specimen(%)

|	Number	Mean sagittal location on articular facet	Sagittal located region on articular facet	Mean sagittal location on physes	Sagittal located region on physes
Femoral ITC	10	58.06 ± 13.66	31.29–84.83	56.92 ± 14.93	27.66–86.18
Anterior tibial ITC	10	29.18 ± 9.26	11.03–47.33	41.10 ± 8.31	24.81–57.39
Posterior tibial ITC	10	56.46 ± 4.87	46.91–66.01	57.74 ± 7.16	43.71–71.77

Note: The mean sagittal location on articular facet and physis were calculated by DAA/(DAA + DPA) and DAP/(DAP + DPP). The sagittal located regions were calculated by the 95% confidence interval (CI) with K-S test.

condylar physeal transgression, respectively. When using a chi-square test to compare the transphyseal OS extension in intercondylar region (33/37) to that in bilateral condylar region (22/33), a significant difference was shown ($\chi^2 = 5.255$, $P = 0.022$).

The above results were consistent with our intuitive observations. Fewer transphyseal tumor extensions were observed in the bilateral condyles than in the intercondylar region (Figure 5A). Meanwhile, in many cases showing the typical ITC-mediated transphyseal OS extension, the inter-condylar transphyseal tumor extension did not traverse the entire epiphysis to reach or breach the articular facet, nor was there coronal-extended penetration of bilateral condylar growth plate; they perforated the growth plates to show an intercondylar "breakpoint" and stopped within the epiphysis (Figure 5B).

Fig. 2 The observation and measurement of ITCs in MRIs and anatomic specimens. (A) The transphyseal vessels (arrowheads) and epiphyseal vascular coalescence (empty arrowhead) in femoral ITC shown on sagittal and coronal MRI. (B) The transphyseal vessels (arrowheads) in anterior tibial ITC shown on sagittal and coronal MRI. (C) The transphyseal vessels (arrowheads) in posterior tibial ITC shown on sagittal and coronal MRI. (D) The number of the femoral and tibial ITC vessels on articular facet and physes ($n = 28$ knees) counted on MRIs. Filled columns for articular facets and empty columns for physes. (E) The distribution of femoral and tibial ITCs along the anteroposterior lines (expressed as percentages) on joint surface shown in frequency distribution histogram. Unimodal distribution for the femoral ITCs, and bimodal distribution for the tibial ITCs. (F) The juxta-articular femoral ITC (arrowhead) and covered synovium (empty arrowhead) shown in anatomical specimen of a 7-year-old girl. (G) The epiphyseal vessel (arrowhead) and walls of bony canal (empty arrowheads) in femoral ITC shown in anatomical specimen of a 7-year-old girl. (H) The entire posterior tibial ITC including the epiphyseal vessel (empty arrowhead) and transphyseal vessel (arrowhead) shown in anatomical specimen of a 14-year-old boy. (I) The count of the femoral and tibial ITC vessels on articular facets and physes in 10 dissections. Filled columns for articular facets and empty columns for physes. (J) Quantitative comparison of the sagittal location (expressed as percentages) of femoral and tibial ITCs determined on MRI, and by direct measurements in anatomic specimens. In comparison, blue boxplots show the location information on articular facets and black boxplots show the location information on physes. (J1) Quantitative comparison of the sagittal location of femoral ITC between MRI and dissection measurements. Boxplots indicate averaged and the minimum/maximum location value of femoral ITCs on articular facets and physes. (J2) Quantitative comparison of the sagittal location of tibial ITC between MRI and dissection measurements. Boxplots indicate averaged and the minimum/maximum location value of tibial ITCs on articular facets and physes. The superior boxplots show posterior ITC, the inferior boxplots show anterior tibial ITC. (K) Angiographic images of the tibial (K1) and femoral (K2) ITC with iohexol being injected into the popliteal vessels, the transphyseal vessels were shown (arrowheads).
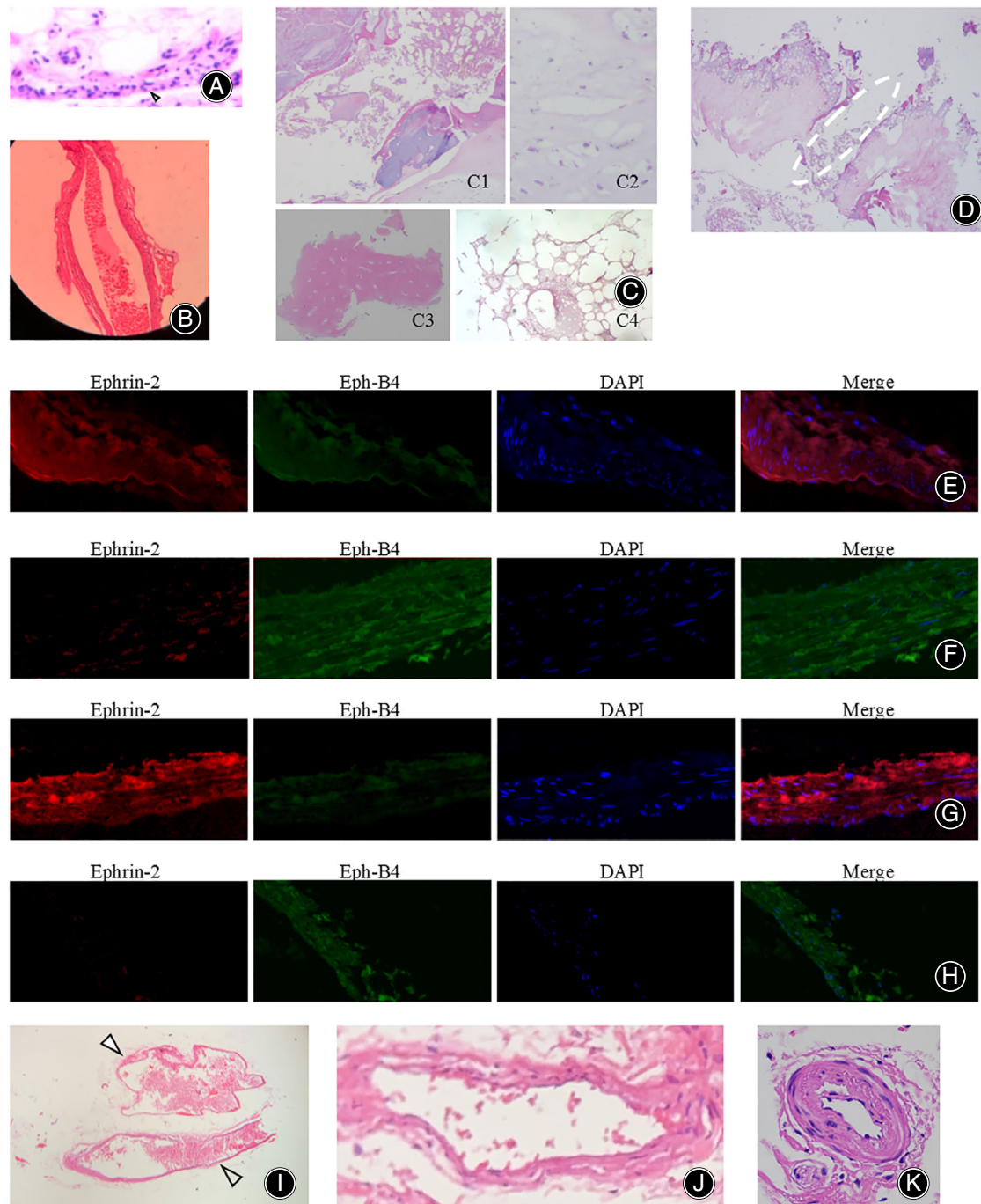
Fig. 3 The histological and molecular characteristics of femoral and tibial ITCs. (A–D) The histological features for the components of ITC revealed by HE staining. (A) Typical synoviocytes (arrowhead) shown in the articular covered soft tissues. (B) ITC vessel with adventitia, media, and intima. (C) The bony canal of ITC in epiphysis (C1) showing cartilaginous tissue (C2), osseous tissue (C3), and bone marrow cells (C4). (D) Transphyseal canal in ITC (dash line) totally interrupted the intact growth plate. (E–H) Vascular identification confirmed by IF. Expression of Eph-B4 (green), ephrin-2 (red), and DAPI (blue). (E) Preferentially positive ephrin-2 expression shown in anterior tibial ITC vessel. (F) Preferentially positive Eph-4 expression shown in posterior tibial ITC vessel. (G,H) Either arterial feature with strongly positive ephrin-2 expression (G) or venous feature with strongly positive Eph-4 expression (H) shown in different femoral ITC vessels. Images (I–K) were performed by HE staining. (I) The vessel with either arterial feature (empty arrowhead) or venous feature (arrowhead) shown in femoral ITC by HE staining. (J) The vessel with venous feature shown in posterior tibial ITC. (K) The vessel with arterial feature shown in anterior tibial ITC.
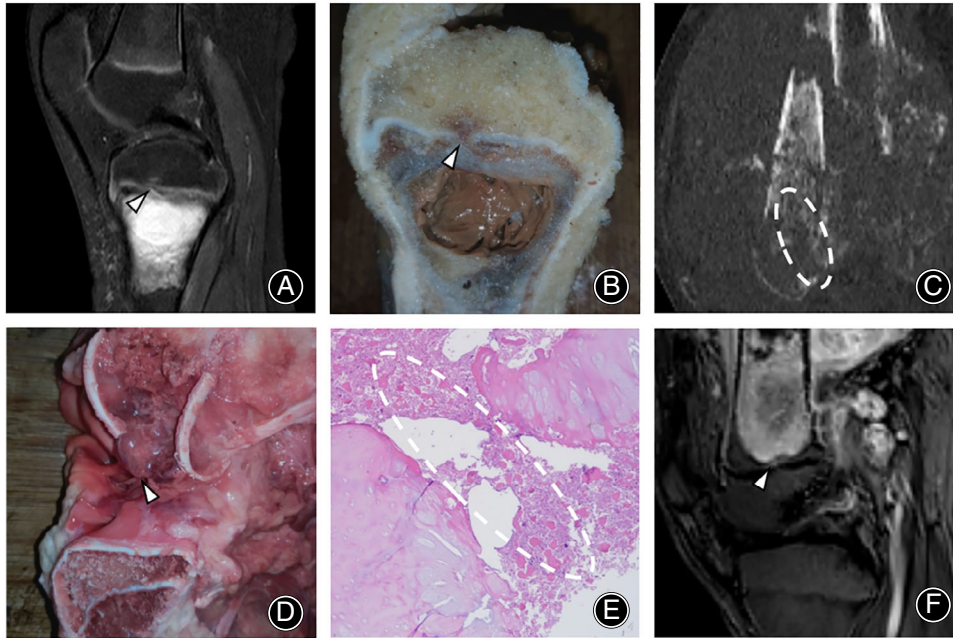
Fig. 4 Tumor extension *via* tibial and femoral ITCs. Images (A) and (B) from a 10-year-old female patient with OS. (A) Transphyseal tumor extension (arrowhead) *via* anterior tibial ITC on MRI. (B) Tumor invasion of the transphyseal site of ITC on growth plate (arrowhead) in gross anatomic view. Images (C–E) from a 14-year-old male patient with OS. (C,D) Tumor extension to articular cavity (arrowhead) from metaphysis *via* femoral ITC (dash line) shown on CT images (C) and anatomical specimen (D). (E) Transphyseal OS spread *via* femoral ITC (dash line) without peripheral cartilaginous destruction, confirmed by pathological examination. (F) Metaphyseal ITC vessel involvement (arrowhead) in OS which occurred in femoral metaphysis without physeal invasion on MRI from a 15-year-old male patient with OS.

Discussion

Normal Morphological Characteristics of ITCs

In this study, the ITC was identified and characterized as a novel transphyseal vasculature-canal compound structure that transgresses the normal growth plate, which is generally believed to possess no transphyseal blood vessels or vascular
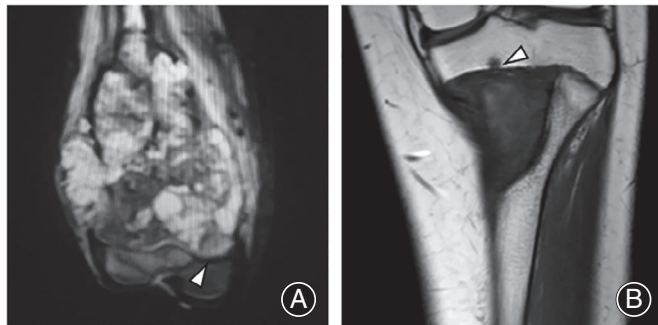
Fig. 5 Osteosarcoma invasion for bilateral condylar and intercondylar physes. (A) Sarcomatous physeal invasion in medial femoral condyle of a 6-year-old girl. The tumor was defended by medial physeal cartilage (arrowhead). (B) Intercondylar tumor transphyseal extension performed as an "breakpoint" transgression on physis (arrowhead) and ceased within epiphysis in a 10-year-old girl.

channels in children over 2 years old[7]. The previous consensus is that the classic transphyseal vessels and vascular channels originating from the periosteum blood vessels will disappear between 12 and 18 months of age, after which the vessels that communicate between the epiphysis and metaphysis will not appear before the growth plate is senescent or closed[7]. In contrast to the previous understanding of transphyseal vessels and canals that terminate in the metaphysis or epiphysis[22,23], the vessels and canals in ITCs traverse the entire epiphysis and growth plate and enter the intraarticular region in the pediatric femur and tibia. ITCs should be inherent in all children with open physes due to such structures being found in all dissections. Furthermore, according to our previous research, the ITC will remain a permanent structure with the corresponding location rather than disappear after the epiphyseal line is closed[5]. The intercondylar foramen in the immature tibia of rodents has been reported by other scholars[24]; however, their investigations focused more on the passage of murine nerves, and transphyseal vessels were not revealed.

Compared with investigations on metaphyseal and epiphyseal vasculatures, previous studies on pediatric genicular vessels are much less common[7,9,25]. Based on our findings, there are two transphyseal vessels that seem to be one artery originating from the MGA branch and one vein originating from the MGV tributary in the proximal tibia and

ITCS AND THEIR ONCOLOGICAL SIGNIFICANCE

distal femur. Such transphyseal sites were observed in all anatomic dissections. Furthermore, we found that the terminal MGA branch and MGV tributary penetrated into the tibia within anterior and posterior tibial ITCs, respectively, rather than entering the intraosseous region concomitantly. This result corresponded with our previous studies in adults[5]. To the best of our knowledge, there are no systematic reports describing the intraosseous extension of middle genicular vessels in the immature tibia and femur.

The discovery of femoral and tibial ITCs may also have relevant physiological significance. First, the continuity of the growth plate with tight cell arrangement and strong germination ability are completely interrupted by ITCs that contain no cartilage cells; therefore, the barrier function of the cartilaginous open physes to lesion invasion may be weakened due to this newly discovered natural gap. Second, the leukocytes were detected in transphyseal ITC vessels, indicating potential nonspecific immunity. Finally, ITCs may also supply some physes or epiphyses.

Radiographical and Pathological Features of ITCs in Pediatric OS Patients

Both radiographical and pathological features of ITCs in pediatric OS patients were preliminary revealed in this study, and high consistency was shown between macroscopic and microscopic observations. Based on these findings, ITCs were proved to play important roles in facilitating the transphyseal osteosarcoma extension through intercondylar physes. This perspective is macroscopically supported by the fact that 100% (12/12) metaphyseal OS transgressed the growth plate in patients with certain ITC tumorous involvements that reach the physeal level. This result showed high accordance with intercondylar transphyseal tumor dissemination in our cross-sectional study. In the HE staining, the transphyseal spread of sarcomatous cells *via* the ITC canal was obvious while the adjacent chondrocytes and extracellular matrix remained intact. In addition, tumor involvement in ITC transphyseal vessels was also found; therefore, both the canal and the vessel in ITC would be regarded as convenient channels that decrease the necessity to generate tumor vessels that degrade the basement membrane and extracellular matrix of physes and subsequently facilitate OS spread[4]. Meanwhile, except for transphyseal ITCs, the other physeal region virtually retains the oncological barrier ability from previous studies. According to these findings, ITC, the "breakpoint" of the ostensibly intact growth plate, may be a risk factor for vulnerability to intercondylar physes, and therefore allows more osteosarcomas to transgress the growth plate.

Clinical Significance of ITCs

To deeply analyze the clinical significance of ITCs in pediatric OS patients, we divided the physis-related lesions into four types in this study. Compared with previous investigations that categorized the degree of OS extension into five types[2], this new classification is more pertinent to evaluate

transphyseal OS extension and to investigate the clinical significance of ITC-mediated tumor dissemination. Our assessments demonstrated that osteosarcoma is more likely to breach the growth plates through the intercondylar region where ITCs are located instead of the bilateral condylar region in distal femur and proximal tibia, implying the vulnerability of intercondylar physes to OS invasion. Moreover, osteosarcomas that could breach the bilateral condylar physeal cartilage appear to have stronger aggressiveness with a more expansive damaged area on the growth plate and possess more opportunities to cause transepiphyseal lesions than the tumors that solely transgress the intercondylar physeal region. As far as we know, this is the first systemic assessment of the discrepancy between the intercondylar and bilateral condylar regions when metaphyseal osteosarcomas invade the physes.

ITC may also play a critical role in more extensive clinical fields. In our opinion, if uninvaded peripheral cartilage is confirmed with MRI and intraoperative pathological examination, ITC ablation may benefit certain OS patients in the following ways. First, less transepiphyseal excision which sacrifices total physes may be chosen due to surgical intervention of the ITCs[1]. Moreover, with the ablation of ITCs, more physeal cartilage may be reserved in transphyseal excision, thus reducing the postoperative growth disturbance and extremity-length discrepancies[26]. However, whether these views are truly suitable primarily for surgical options will depend on future scientific clinical trials to evaluate oncologic and functional outcomes. In addition, some scholars reported that transphyseal spreading of pyogenic infection may occur in 81% of patients aged 2–16 years[27]. Thus we hypothesize that ITC may participate in transphyseal osteomyelitic spreading as a media. Therefore, the findings of ITC may have broader applications in knee-related diseases.

Limitations of this Study

It has to be acknowledged there existed several limitations in this study. First, the interlayer artifacts produced by MRIs and unknown vascular malformations may influence the observation of ITCs. Thereby, radiographs with higher resolution may help to deeply analyze the microarchitectures of the ITCs in following investigations. Second, although we located all the ITCs in intercondylar fossa and tibial intercondylar regions, the coronal range for the ITC was not geometrically expressed due to the transphyseal sites located in quite wide regions in coronal direction according to our observations. Finally, more sophisticated studies are required to elaborate on how ITCs mediate the intercondylar transphyseal tumor extensions on the level of molecular biology; therefore, multicenter and long-term investigations are warranted in the future.

Conclusion

In this research, we discovered a novel vasculature-canal compound structure, ITC, and investigated its potential

oncological significance. ITCs are previously undiscovered channels that interrupt the tight chondrocyte arrangement of the growth plate. As such unfound "gaps" on ostensibly intact open physes, ITCs are deemed to facilitate the OS to transgress the physeal barrier, and perhaps induce the vulnerability of intercondylar growth plates when defending against OS invasion. Our discovery and characterization of ITCs may promote the understanding of pediatric intra-osseous vessels, growth plates, and transphyseal OS extension. With further elaboration of this structure, surgical and adjuvant treatment strategies may be ameliorated for pediatric osteosarcomas.

Supporting Information

Additional Supporting Information may be found in the online version of this article on the publisher's web-site:

Table S1. Demographic and lesion type of 38 patients

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