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Histogenesis of clear cell chondrosarcoma *

An immunohistochemical study with osteonectin, a non-collagenous structure protein

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Summary. The histogenesis of clear cell chondrosarcoma is still unclear: Apart from typical clear cell tumor areas, extensive production of woven bone formation suggests within the clear cell cartilagineous stroma is an intriguing phenomenon. Three cases of clear cell chondrosarcoma documented in the Bone Tumor Registry of Westphalia were examined for their patterns of osteonectin expression, and compared with other bone tumors of either osseous or cartilagineous origin, and with normal cartilage tissue. Found predominantly in osseous structures, the protein osteonectin takes part in the formation of new bone. The three clear cell chondrosarcomas showed a strong immunoexpression of osteonectin in clear cell, chondroid and in osseous tumor areas. Similarly, evidence of osteonectin was also found in osteoblastic and in chondroblastic osteosarcomas as well as in osteoblastomas. In contrast, osteonectin could not be demonstrated in the chondrosarcomas and mesenchymal chondrosarcomas from our registry that were analysed for comparison, and was found only minimally in the fibroblastic areas of dedifferentiated chondrosarcomas. The chondroblastic tumor components were always negative. There was no immunoexpression of osteonectin either in fetal or adult intervertebral disc tissue. The present immunohistochemical study of osteonectin has distinctly separated clear cell chondrosarcoma from the other variants of chondrosarcoma, and aptly verified the specificity of this entity. Moreover, the study would call for further histogenetic evaluation of clear cell chondrosarcoma, since the pattern of osteonectin expression in that tumor seems to indicate an osteogenic rather than a chondrogenic origin.

Key words: Clear cell chondrosarcoma – Immunohistochemistry – Osteonectin – Osteosarcoma

* Dedicated to Professor Günther Schellong on the occasion of his 65th birthday, January 15, 1991

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Introduction

Clear cell chondrosarcoma, first described by Unni et al. in 1976, is distinguished from classical chondrosarcoma by its typical histological picture, its mostly epiphyseal site of origin, and its relatively benign clinical course (Unni et al. 1976). Several criteria indicate that this rare lesion might be a subtype of chondrosarcoma, but the histogenetical and etiological classification is as yet unsatisfactory. Beside the typical clear cell tumor areas, some cell-rich regions, with numerous osteoid trabeculae are regularly observed, suggesting an osteogenic rather than a chondrogenic origin. Moreover, the frequent evidence of many multinuclear giant cells of osteoclast type cannot be taken as a sign of chondrogenic origin either, because these are generally absent in classical chondrosarcoma (Björnsson et al. 1984). Electron microscopy, too, fails to provide definite criteria for etiology: the ultrastructural changes observed in clear cell chondrosarcoma – cytoplasmic microvilli, a large amount of glycogen particles, and prominent Golgi complexes – are equally present in definitely osteogenic tumors (Charpentier et al. 1979; Angervall et al. 1980, Grundmann et al. 1981).

Recently, the perfection of immunohistochemical methods has been of great value for a better histo-etiological distinction and classification of bone tumors (Cavazzana et al. 1987; Brooks and Trojanowski 1987; Roesner et al. 1989). Antibodies directed against non-collagenous structure proteins are now available, providing a new approach to the etiology and the histological classification of clear cell chondrosarcoma on an immunohistochemical level.

Osteonectin, one of the major non-collagenous structure proteins, occupies some 10% of organic bone matrix (Termine et al. 1987). It is a phosphorylated glycoprotein of 38 kilodalton molecular weight that was first isolated from bovine bone tissue (Termine et al. 1981). Osteonectin affects the organisation and mineralisation of bone matrix, takes part in calcium metabolism, and shows a high affinity to collagen (Doi et al. 1989; Maniatopoulos

Table 1. Clinical data of the three clear-cell chondrosarcomas

Case	Age (years)	Sex	Localization
1	37	M	Epiphysis proximal humerus
2	60	F	Epiphysis of femur
3	48	M	Metaphysis proximal femur

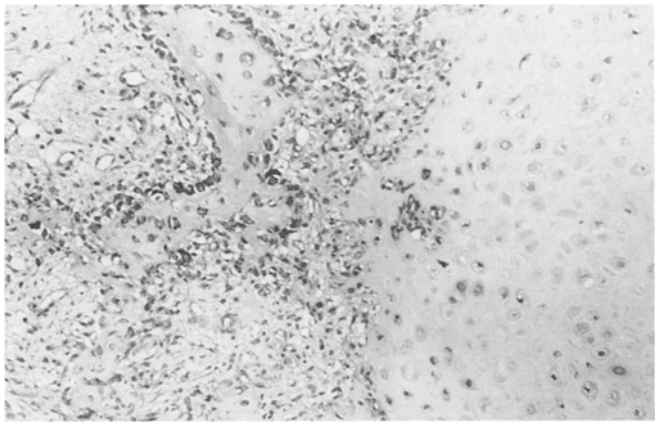

Fig. 1. Fresh human callus serving as positive control: strong immunoexpression in the cuboidal osteoblasts, negative immunoexpression in the chondroid area (anti-osteonectin APAAP 120×)

et al. 1988; Kuboki et al. 1989). The demonstration of osteonectin DNA sequences, too, confirms its high affinity to calcium and to hydroxyapatite (Bolander et al. 1988). The chemical and binding capacities of osteonectin are similar to those of the recently reported "SPARC" (Domenicucci et al. 1988; Howe et al. 1988). In fact, osteonectin appears to be a multifunctional protein primarily found in osseous structures (Bianco et al. 1988). It does occur in several benign and malignant boneforming tumors, but also in the osteoblasts of reactive bone lesions. It was rarely found in chondrosarcomas except for tumor cells intimately associated with chondroosteoid (Jundt et al. 1987; Schulz and Jundt 1989; Bosse et al. 1990).

The three cases of clear cell chondrosarcoma documented in the Bone Tumor Registry of Westphalia, were analysed with antibodies against osteonectin in addition to conventional histomorphological investigation, in an attempt to evaluate their true histo-etiological position. For controls and comparison we used several documented bone tumors of definite osseous or cartilaginous origin, as well as specimens of normal chondroid tissue.

Material and Methods

The study comprises three clear cell chondrosarcomas, nine chondrosarcomas (GI–GIII), two mesenchymal chondrosarcomas, three dedifferentiated chondrosarcomas, five osteoblastic osteosarcomas, five chondroblastic osteosarcomas, five osteoblastomas, and two surgical specimens of fetal and two of adult hyaline cartilage tissue.

Primary antibodies were kindly supplied by Drs. Termine, Geron-Robey and Fisher of the National Cancer Institute, Bethesda/Md. These were raised in rabbits against bovine and human osteonectin as described by Termine et al. (1981). The specificity of these antibodies was checked by Ouchterlony tests. All our immunohistochemical investigations were performed on formol-fixed material (4% buffered formol) embedded in paraffin. A major part of the sample was already decalcified with 10% EDTA solution. For demonstration of osteonectin we used the modified alkaline phosphatase-antialkaline phosphatase (APAAP) method with an extra incubation step by inserting an additional mouse-anti-rabbit Ig antibody (1:125, Dako, Hamburg) between the primary and the link antibody (Cordell et al. 1984). Deparaffinated sections were incubated for 30 min with the primary antibody in a 1:400 solution. Trypsinase pretreatment was without influence on the immunocytochemical reaction, which yielded identical results for human and bovine osteonectin. The immunohistochemical demonstration of osteonectin in fresh callus tissue was taken as positive control (Fig. 1). Negative controls were established by omitting the primary antibody, or by using normal rabbit serum. The link antibody (rabbit-anti-mouse Ig 1:30) and the APAAP complex (1:100) were also kept on the slides for 30 min each (both from Dako, Hamburg). All incubation steps alternated with 5 min through washing of samples with Tris buffer. Color development required 30 min incubation in naphthol AS-BI phosphoric acid/dimethyl formamide. Simultaneously, endogenous alkaline phosphatase was suppressed by levamisole.

Results

In conventional histology, the three *clear cell chondrosarcomas* show mainly the typical clear cell tumor areas; the cytoplasm of the tightly packed large cells appears either eosinophilic or optically empty. Tumor cells with a slightly microlobular pattern are separated by a slender fibrovascular stroma wherein numerous vessels are embedded. Mitoses are infrequent (Fig. 2a). In two of our cases, some foci were found to reveal the classic features of moderately differentiated chondrosarcoma cells with small, often elongated nuclei, embedded in chondroid ground substance. Appearing only sporadically, such areas were never predominant in quantity (Fig. 2b). A regular occurrence were large areas with cell-rich vascular stroma enclosing newly formed bone trabeculae and carrying broad osteoblastic rims. Small groups or disseminated multinuclear giant cells of osteoclast type were often seen in between, but no chondroid or clear cell tumor portions whatsoever were seen in these regions (Fig. 2c). In other parts, newly formed bone trabeculae in a loose meshwork were demonstrable within the clear cell tumor component.

Immunohistochemical analysis of clear cell chondrosarcoma with anti-osteonectin reveals a strong immunoexpression of osteonectin with marginal intracytoplasmic accentuation within the clear cell tumor areas. Some cells, however, are negative (Fig. 3a). The scattered chondrosarcomatous tumor areas are also positive for osteonectin (Fig. 3b). The osteoblastic tumor component, too, has a markedly positive immunoexpression for the osteoblastic rim. Intermediate zones, in contrast, are negative, and so are the multinuclear giant cells (Fig. 3c).

A marked positive immunoreaction appears in the osteoblastic tumors. Classic *osteoblastic osteosarcomas* express osteonectin mainly in the osteoblasts near newly formed trabeculae (Fig. 4a). *Chondroblastic osteosarcoma* shows a strong immunoexpression in its cartilage cell (chondrocytic) regions (Fig. 4b). *Osteoblastoma* reveals a

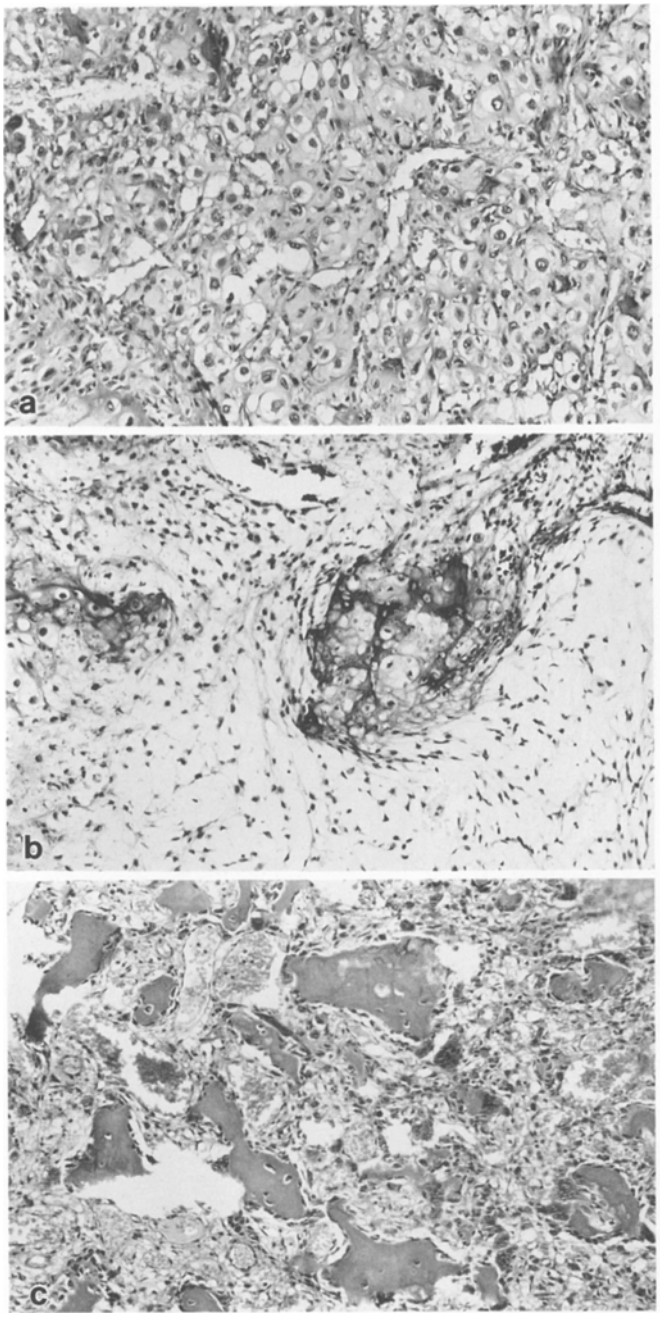

Fig. 2 a–c. Different aspects of clear-cell chondrosarcomas: **a** typical clear-cell tumour area with lobular cell complexes with moderate nuclear polymorphism and honycombed cytoplasm; **b** chondrosarcomatous tumour area with clear-cell insular cell complexes; **c** osseous tumour component with partly mature bone trabeculae with broad osteoblastic rims, many blood vessels and multinucleate giant cells. H & E 120 ×)

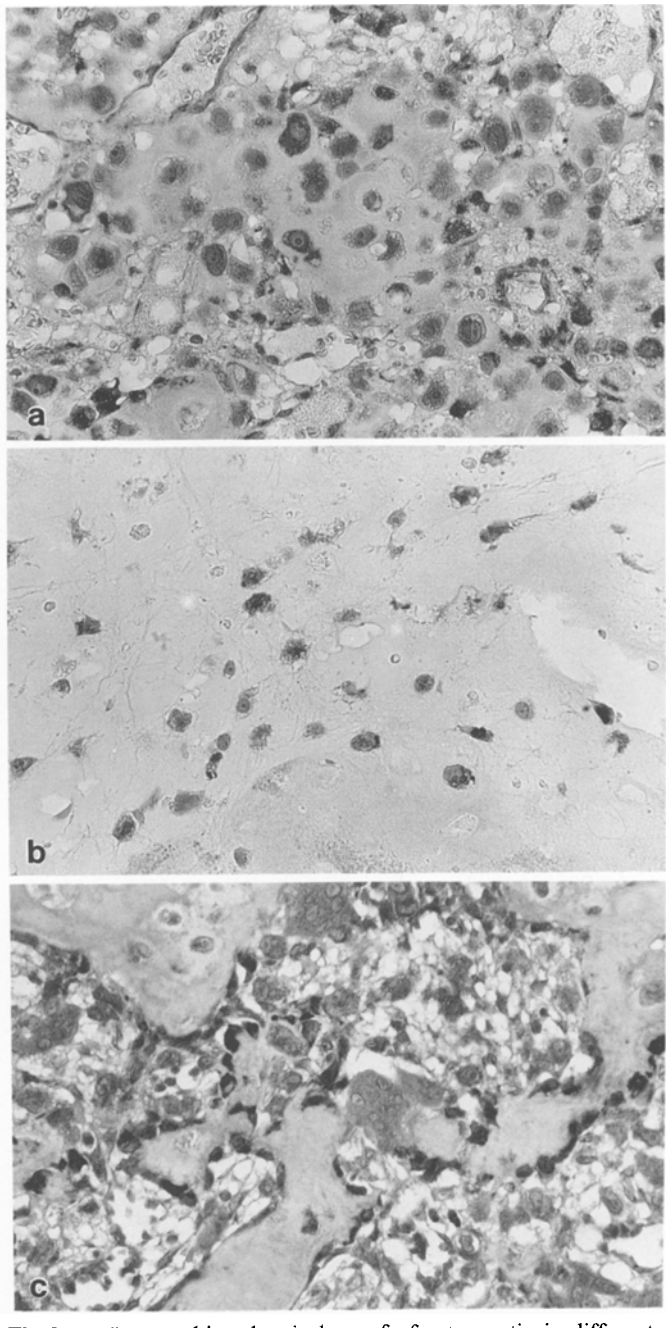

Fig. 3 a–c. Immunohistochemical proof of osteonectin in different parts of clear-cell chondrosarcoma with strong immunoreaction pattern in the cytoplasm of the typical clear cells (**a**), in the chondrosarcomatous tumour cells (**b**), and in the prominent osteoblasts of the osseous areas (**c**) (all anti-osteonectin APAAP 360 ×)

pronounced positivity on the osteoblastic rim (Fig. 4c). In contrast, no positive immunoreaction can be established in the *chondrosarcomas* (grades I–III) analysed for comparison; there was only a very slight positivity in some areas of osteoid trabeculae. In *mesenchymal chondrosarcoma* the immunoreaction is entirely negative for osteonectin. *Dedifferentiated chondrosarcomas* (grade IV) carries some scattered positive cells in its dedifferentiated fibroblastic areas, but here, too, the chondromatous tumor component is definitely negative without any evidence of osteonectin expression (Fig. 5a–c).

In both control samples – fetal cartilage of the intervertebral disc with central residues of notochord, and adult cartilage of the intervertebral disc – no osteonectin can be demonstrated (Fig. 6a, b). All results are listed in Table 2.

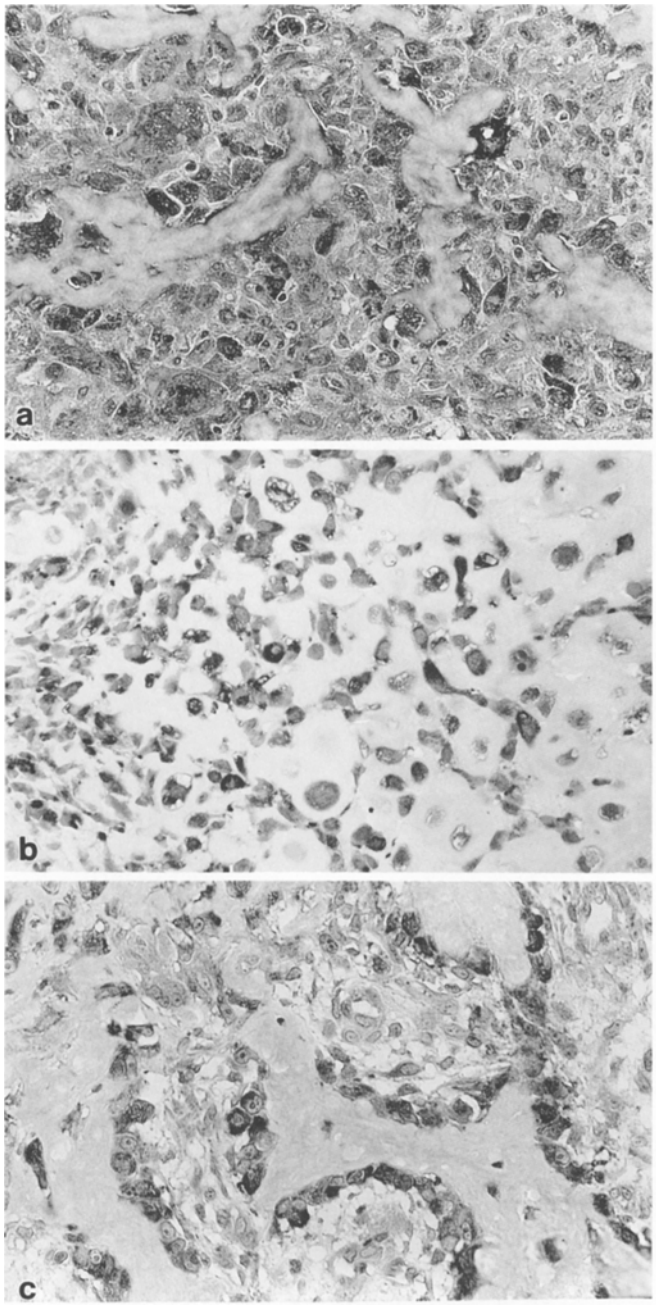

Fig. 4 a–c. Immunohistochemical results of osteonectin in osteoblastic osteosarcoma with strong immunoreaction pattern in osteoblasts near trabeculae (**a**), in a chondroblastic osteosarcoma with moderate to faint positive labelling of nearly all tumour cells (**b**), in an osteoblastoma with positive reaction in the proliferating osteoblasts (**c**) (all anti-osteonectin APAAP 360 ×)

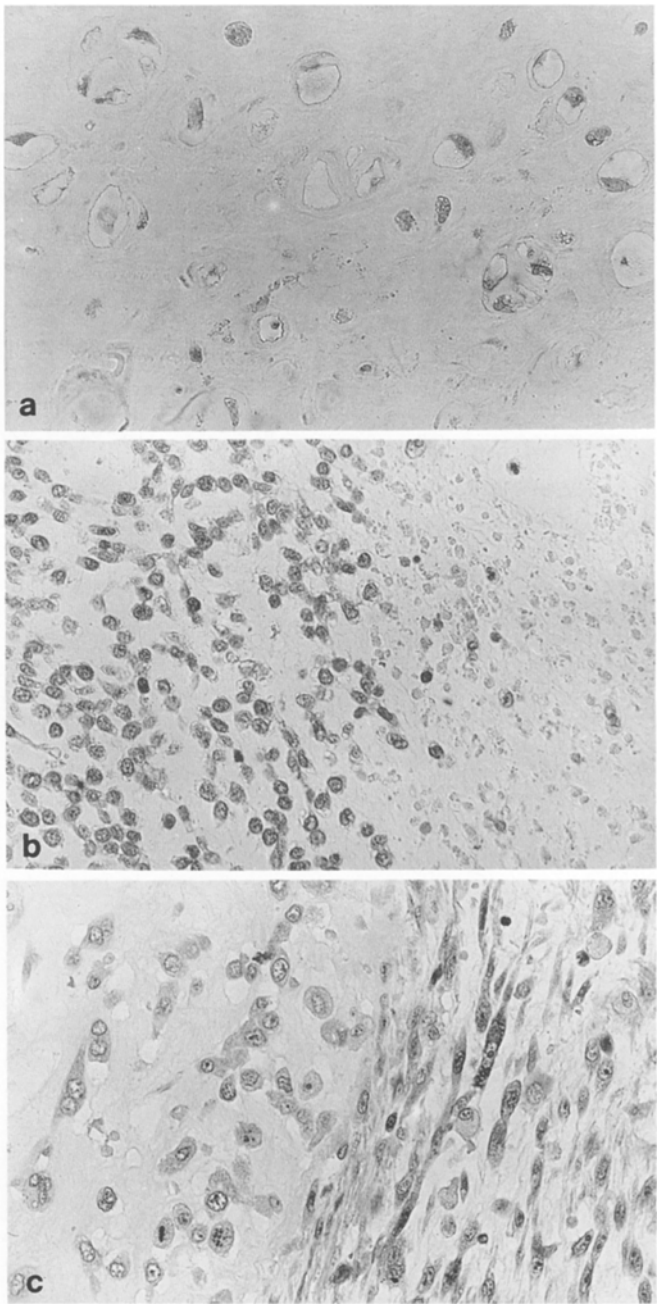

Fig. 5 a–c. Immunohistochemical results of osteonectin in chondrosarcomas at different levels of differentiation. Completely negative immunoreaction in a highly differentiated chondrosarcoma (**a**), and in a mesenchymal chondrosarcoma (**b**). One positive cell in a dedifferentiated chondrosarcoma in the spindle-shaped cells, the chondromatous tumour component is completely negative (**c**) (all anti-osteonectin APAAP 360 ×)

In the same way fresh callus tissue, taken as positive control, reveals osteonectin-negative cartilage cell areas with but a few scattered positive chondrocytes in the immediate vicinity of positive osseous regions (Fig. 1).

Discussion

Clear cell chondrosarcoma (CCCS) has been controversially discussed since the very first description as a distinct clinical and pathological entity (Unni et al. 1976). Although the lesion clearly shows a more benign course than typical chondrosarcoma, its true histogenesis has not yet been fully ascertained. The pathological features that seem to distinguish it from genuine osseous tumors were somewhat blurred by the "bimorphic" histology suggesting both chondroid and osseous origin. Obviously the regular evidence of newly formed osteoid trabeculae, of multinuclear giant cells (osteoclast type), and the

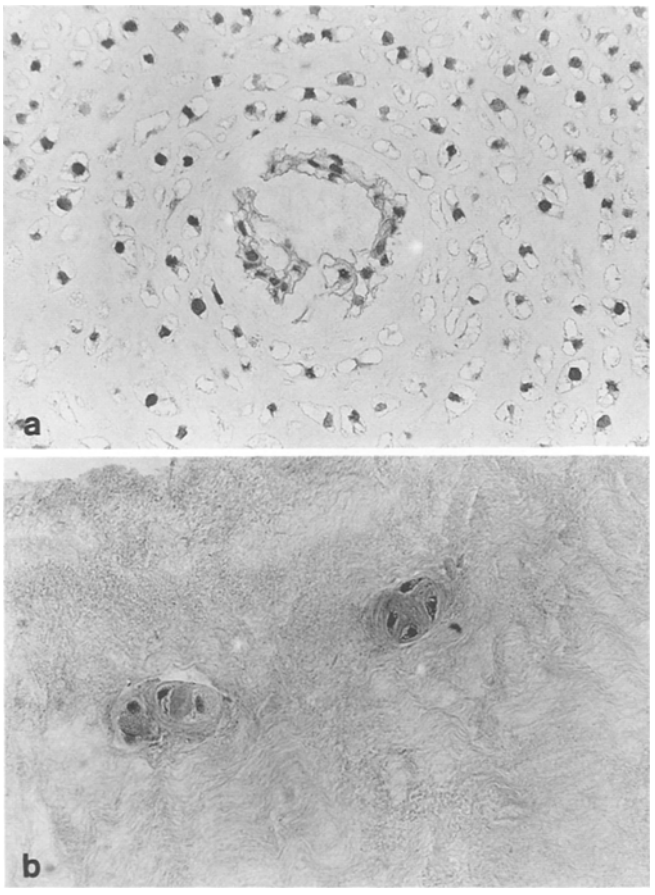

Fig. 6 a, b. Completely negative immunoreactions with anti-osteonectin in the cartilage cells of a fetal vertebral disc with central residues of noto chorda (**a**), negative immunoreactions in the cartilage cells of the vertebral disc of an adult (**b**). (All anti-osteonectin APAAP, 360× in **a**, 550× in **b**)

Table 2. Immunohistological results with osteonectin antibody in clear-cell chondrosarcomas, bone tumours of osseous and cartilage origin and normal chondroid tissue

Material	No. of cases	Anti-osteonectin reaction intensity[a]
Clear-cell chondrosarcoma	3	+++
Osteoblastic osteosarcoma	5	+++
Chondroblastic osteosarcoma	5	+++
Osteoblastoma	5	++
Chondrosarcoma (GI–GIII)	9	–[b]
Mesenchymal chondrosarcoma	2	–
Dediff. chondrosarcoma	3	+[c]
Fetal cartilage	2	–
Adult hyaline cartilage	2	–

[a] +, low; ++, moderate; +++, strong
[b] Positive immunoreactivity occurring only in areas of osteoid trabeculae
[c] Positive immunoreactivity occurring only in the dedifferentiated tumour areas

mostly epiphyseal localisation would contradict a truly chondrosarcomatous source. Electron microscopic investigations also failed to supply definite indications of histogenesis: the ultrastructural alterations documented in clear cell chondrosarcoma, such as cytoplasmic microvilli, numerous glycogen particles, and prominent Golgi complexes, may also be found in osteogenous tumors (Grundmann et al. 1981).

Immunohistochemical techniques provided a new tool for establishing the histogenesis of many malignant tumors. A neural differentiation of Ewing's sarcoma, for example, in certain conditions could be ascertained and verified by these methods (Cavazzana et al. 1987), and chondroid chordoma was identified as a low-grade chondrosarcoma on the immunohistochemical level (Brooks et al. 1989). Further, immunohistochemistry succeeded in establishing the cytogenesis of macrophages and osteoclast-like giant cells in several bone tumors (Roessner et al. 1989). Concerning clear cell chondrosarcoma, Weiss and Dorfman (1986) had demonstrated intense immunoreactivity of tumor cells for S 100 protein, conclusive, in their opinion, of the cartilagineous origin of that tumor. However, we cannot necessarily believe S 100 protein to be a marker for cartilagineous origin: Recent studies have verified a wide distribution of S 100 protein in other than nerval or cartilagineous tissues, and it was also demonstrated in chondroblastic osteosarcoma (Nakamura et al. 1983; Haimoto et al. 1987).

Antibodies directed against non-collagenous structure proteins, which are now available, promised a new approach, too, for demonstrating the origin and histogenesis of clear cell chondrosarcoma.

Of these structure proteins, the most important and so far, the most readily accessible type for physical characterisation is *osteonectin* (Tracy et al. 1988). One of its most intriguing factors is the ability to bind closely to hydroxyapatite (Termine et al. 1981) and to collagen; it is also a potent growth inhibitor of calcium phosphate crystals (Romberg et al. 1986). During mineralisation, osteonectin may act as a regulator of hydroxyapatid formation on collagen fibrils. Whenever several different tissues were studied side by side, osteonectin was found primarily associated with bone in most cases (Termine et al. 1981; Jundt et al. 1987; Schulz and Jundt 1989). It has been shown, however, that osteonectin does not occur exclusively in bone-forming tumors, but that if present it will always be found close to bone trabeculae and mineralisation zones (Bosse et al. 1990). With regard to the distribution of osteonectin in benign and malignant cartilagineous tumors other than clear cell chondrosarcoma, previous immunohistochemical studies have shown that it was rarely present in tumor tissue, except in tumor cells intimately associated with chondroosteoid (Schulz and Jundt 1989; Bosse et al. 1990).

Our present study yielded almost similar findings: Osteonectin was demonstrated not in cartilagineous, but only in osseous areas of fetal and adult skeletal tissues. In bone tumors, the strong positive immunoreaction was evident in both benign and malignant bone-forming tumors such as osteoblastoma, osteoblastic and chondroblastic osteosarcoma. Conventional and mesenchymal chondrosarcoma failed to show positive reactions for osteonectin except in tumor cells intimately associated with chondroosteoid structures. Although an anaplastic tu-

mor area of dedifferentiated chondrosarcoma would sometimes show a positive reaction for osteonectin, its chondroid component was always negative.

In contrast, within the clear cell chondrosarcomas studied here, osteonectin was demonstrated not only in the areas of osteoblastic differentiation, but also distinctly in areas of purely chondrosarcomatous histology which had supported its classification as a variant of chondrosarcoma (Unni et al. 1976).

The strong immunoexpression of osteonectin in our three clear-cell chondrosarcomas fails to correspond with the osteonectin pattern in tumors of chondroid differentiation, nor does it agree with that in normal chondroid tissue. It was shown that clear cell chondrosarcoma are practically indistinguishable from bone tumors in terms of osteonectin immunoexpression.

These results suggest that the regular evidence of bone matrix in clear cell chondrosarcoma would indicate an osteogenic rather than a chondroid origin. As early as 1925, Codman had based his definition of osteosarcoma of the leading criterium of osteoid formation: "Sarcoma derived from tissue presumably intended to form bone, irrespective of whether it eventually does so" (Codman, 1925). On the basis of this leading concept, Ewing had defined chondrosarcoma as a separate entity to be distinguished from osteosarcoma (Ewing 1935).

According to Codman's classification, clear cell chondrosarcoma should be seen as a bone tumor even by histomorphological criteria alone. The present immunohistochemical study which demonstrated the osteoblastic nature of clear cell chondrosarcoma not only in its osteoblastic, but also in its clear cell and even its cartilaginous areas, provides further support for the proposed osseous origin of the tumor.

Other interpretations, however, should also be considered for the intense expression of osteonectin in clear cell chondrosarcoma, since recent publications have proposed some functional role of osteonectin other than that in the mineralization of bone. Wewer et al. (1988) demonstrated the expression of osteonectin in decidual cells, and in poorly differentiated adenocarcinoma cells, suggesting its possible function in active cell proliferation and the remodelling of tissues. In our context, that possibility appears rather less likely, clear cell chondrosarcoma being a tumor of low-grade malignancy that shows no active proliferation of remodelling of tissue.

That osteonectin contributes to the mineralization of epiphyseal cartilage has been shown by Metsäranta et al. (1989) and Pacifici et al. (1990). It is abundantly expressed, both on RNA and protein levels, by hypertrophic chondrocytes, and is deposited in the matrix of mineralizing hypertrophic cartilage. That intracellular immunoreaction for osteonectin was very weak in these cells may be explained by the rapid secretion of that substance. The possibility of a hypertrophic chondrocyte-like differentiation of clear cell chondrosarcoma cells cannot be definitely excluded, since a disturbed secretion of certain products is known to occur sometimes in the process of malignant transformation (Wewer et al. 1989; Ueda et al. in press). The idea might be pursued further by the analysis of substances typical of hypertrophic chondrocytes, such as collagen type X and chondrocalcin, in clear cell chondrosarcoma (Schmid and Linsenmayer 1985; Poole et al. 1984).

We may conclude that our immunohistochemical study on osteonectin has served to separate clear cell chondrosarcoma distinctly from the other types of chondrosarcomas, and to verify the specificity of the entity that had first been supposed by Unni et al. in 1976 on account of its characteristic clinicopathological features. As regards their expression of osteonectin, the tumor cells of clear cell chondrosarcoma observed in its clear cell, its chondrosarcomatous, and also in its osteogenic area, are more closely related to osteoblastic than to chondrogenic cells, a most interesting feature. Thus, further investigation and histogenetic evaluation of clear cell chondrosarcoma is indicated.

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