American Journal of Pathology, Vol. 150, No. 6, June 1997

Phenotypic Diversity of Neoplastic Chondrocytes and Extracellular Matrix Gene Expression in Cartilaginous Neoplasms

Thomas Aigner,* Susanne Dertinger,* Silvia Isolde Vornehm,† Jayesh Dudhia,‡ Klaus von der Mark,† and Thomas Kirchner*

From the Institute of Pathology and the Institute of Experimental Medicine and Connective Tissue Research,† University of Erlangen-Nürnberg, Erlangen, Germany; and the Kennedy-Institute of Rheumatology,‡ Bute Gardens, Hammersmith, London, United Kingdom*

Chondrocyte differentiation is characterized by distinct cellular phenotypes, which can be identified by specific extracellular matrix gene expression profiles. By applying* in situ *analysis on the mRNA and protein level in a series of benign and malignant human chondrogenic neoplasms, we were able to identify for the first time different phenotypes of neoplastic chondrocytes* in vivo*: 1) mature chondrocytes, which synthesized the characteristic cartilaginous extracellular tumor matrix, 2) cells resembling hypertrophic chondrocytes of the fetal growth plate, 3) cells resembling so-called dedifferentiated chondrocytes, and 4) well differentiated chondrocytic cells, which expressed type I collagen, indicating the presence of post-hypertrophic differentiated neoplastic chondrocytes. Chondrocytes exhibiting a range of phenotypes were found to be present in the same neoplasm. The different observed phenotypes, including the dedifferentiated phenotype, were in contrast to the anaplastic cells of high-grade chondrosarcomas. Comparison of expression data with tumor morphology revealed a relationship between the cellular phenotypes, the tumor matrix composition, and the matrix and cell morphology within the neoplasms. The distinctly different phenotypes of neoplastic chondrocytes are the basis of the characteristic high biochemical and morphological heterogeneity of chondroid neoplasms and shed light on their biological and clinical behavior. (Am J Pathol 1997, 150:2133–2141)

Analysis of extracellular matrix gene expression pattern, in particular subtyping of collagen gene expression, has been shown in numerous studies to be a potent and reliable tool to define different phenotypes of chondrocytic cells in the fetal growth plate *in vivo* and in various culture systems *in vitro* (for review see Refs. 1 and 2). Thus, chondroprogenitor cells are characterized by their specific gene product, the alternative splice variant of type II collagen COL2A.[3,4] Mature chondrocytes express the typical cartilage collagen types II (COL2B), IX, and XI as well as aggrecan and link protein.[4–6] These gene products are not specific for cartilage, but they are limited to only a few other tissues such as the vitreous body.[7,8] Hypertrophic chondrocytes are marked by their unique gene product, type X collagen. These cells are solely found in the lowest zone of the fetal growth plate cartilage[9,10] and in other sites of ongoing endochondral ossification such as osteophyte and fracture callus formation.[11,12] Chick chondrocytes have been shown to be able to undergo post-hypertrophic differentiation to osteoblast-like cells, which express specifically type I collagen.[13–15] Another phenotype, which was so far only described *in vitro*, are the so-called dedifferentiated chondrocytes. They are of typical spindle-like or stellate cell shape and synthesize collagen types I and III but not the cartilage typical collagen subtypes nor aggrecan proteoglycan.[16–19]

Several studies have analyzed the behavior of non-neoplastic[16,17,19,20] and transformed or immortalized chondrocytes[21–23] *in vitro*. At present, no analysis exists of the differentiation pattern of neoplastic chondrocytes *in vivo*. There is also little information about development, biochemistry, and cell

Supported by the Thyssen Foundation (Thyssen grant AZ 1993/1/74).

Accepted for publication February 13, 1997.

Address reprint requests to Dr. T. Aigner, Institute of Pathology, University of Erlangen-Nürnberg, Krankenhausstrasse 8–10, D-91054 Erlangen, Germany.

biology of cartilaginous tumors. In one systematic biochemical study, Mankin et al[24,25] analyzed the collagen, proteoglycan, and water content in a variety of cartilaginous tumors and concluded that they qualitatively resemble original cartilage. Mankin et al stressed the striking heterogeneity between the different tumors investigated but also a correlation between biochemical composition and tumor morphology. Few and, in part, conflicting immunohistochemical results exist about the exact tissue composition of cartilaginous neoplasms.[26,27]

In this study, we have investigated mRNA expression and protein distribution patterns of various collagen subtypes as well as other cartilage matrix components *in vivo* in a series of the most frequent benign and malignant subtypes of cartilage-forming neoplasms (chondromas and conventional chondrosarcomas). We were able to extend previous biochemical and immunohistochemical data on the extracellular tumor matrix composition of chondrogenic tumors and specify different cellular phenotypes within these neoplasms.

Materials and Methods

Specimens and Tissue Preparation

Seven cases of chondromas and fourteen cases of (conventional) chondrosarcomas were analyzed. The latter were graded according to standard criteria such as matrix appearance, cellularity, mitotic activity, and nuclear polymorphism as low (grade I; n = 8), intermediate (grade II; n = 4), and high grade (grade III; n = 2).[28] For comparison, four samples of fetal growth plate cartilage (femoral condyles and heads) were included in the study and processed in parallel. Specimens were routinely fixed and decalcified in 0.3 mol/L EDTA (pH 7.5) before embedding in paraffin. Five-micron sections were cut and stored at room temperature until use.

Histochemical Detection of Collagens and Proteoglycans

The collagen content of the extracellular tumor matrix was estimated by Masson-Goldner's and van Giesons stains. Toluidine blue and safranin O staining was performed on parallel sections to estimate the content of proteoglycans.[29]

Immunohistochemistry

Deparaffinized sections were incubated with testicular hyaluronidase (2 mg/ml in phosphate-buffered saline (PBS), pH 5, for 60 minutes at 37°C) and pronase (2 mg/ml in PBS, pH 7.3, for 60 minutes at 37°C) or protease (0.02 mg/ml in PBS, pH 7.3, for 60 minutes at 37°C). Primary antibodies were incubated overnight at 4°C and visualized using alkaline-phosphatase-labeled secondary antibodies and naphthol-X-phosphate and fast red (both from Sigma, Poole, UK) as color substrates.

Nuclei were counterstained with hematoxylin. Alternatively, peroxidase-labeled secondary antibodies and 3,3′-diaminobenzidine as color substrate were used (detection of S-100 protein).

Rabbit antisera against human type I collagen were prepared as described elsewhere.[30] Monoclonal antibodies against chick type II collagen (CIID3), which were shown to cross-react to human type II collagen, were kindly provided by Dr. R. Holmdahl.[31] Polyclonal antibodies against human type III procollagen were kindly provided by Dr. Günzler (Hoechst Co., Frankfurt, Germany). Monoclonal antibodies against human type X were prepared as described elsewhere.[32] Polyclonal antibodies to S-100 protein were purchased from Dako (Glostrup, Denmark).

Preparation of RNA Probes

For specific RNA probes, suitable fragments of human collagen chains α1(I), α2(I), α1(II), α1(III), α1(IX), α1(X), α1(XI), aggrecan, and link protein mRNA were selected and recloned into pGEM/pBS vectors (Promega, Madison, WI, and Stratagene, La Jolla, CA) and (α-^{35}S)UTP-labeled riboprobes synthesized as described elsewhere.[4,9,33]

pHCG 1N contains a 207-bp amino-terminal fragment of collagen α1(I),[33] pHCG 2 a 435-bp carboxyl-terminal fragment of collagen α1(II),[33] which recognized all splicing variants of type II collagen (COL2A and COL2B), pHCG 3 a 294-bp carboxyl-terminal fragment of collagen α1(III),[33] pHCG 9A1L a 941-bp fragment of the first six exons specific for the cartilage splicing variant of collagen α1(IX),[4] pER X a 329-bp carboxyl-terminal fragment of collagen α1(X), and pHCG 11A1 a 351-bp fragment of collagen α1(XI).[4] pKS H4 contained a 1.6-kb fragment of human aggrecan core protein,[34] and pKS8.1D3 contained a 1.9-kb fragment of human link protein.[35]

pRNA 1 contains a 294-bp fragment of mouse 18 S rRNA.[36] This probe shows 100% homology to human 18 S rRNA and was used as a positive control for preservation of the RNA content in the samples during the technical procedures.

In Situ *Hybridization*

In situ hybridization was performed as described in detail elsewhere.[36] Briefly, deparaffinized and rehydrated sections were digested 7 minutes with proteinase K (20 μg/ml in 50 mmol/L Tris/HCl (pH 8) and 5 mmol/L EDTA at room temperature), post-fixed for 5 minutes with 4% paraformaldehyde (in PBS), washed briefly in double-distilled water, acetylated for 10 minutes in 0.25% acetic acid anhydride (in 0.1 mol/L triethanolamine, pH 8), washed again in PBS and double-distilled water, and dehydrated. The sections were hybridized for 12 to 16 hours at 43°C with riboprobes at a final activity of 3×10^7 to 6×10^7 cpm/ml depending on their length. The hybridization buffer contained 50% formamide, 10% dextran sulfate, 20 mmol/L dithiothreitol, 1 mg/ml tRNA, 300 mmol/L NaCl, 10 mmol/L Tris/HCl (pH 7.4), 10 mmol/L NaH_2PO_4 (pH 6.4), 5 mmol/L EDTA, 0.02% Ficoll 400, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin. After hybridization, the tissue sections were washed at 40°C in 2X standard saline citrate (SSC)/0.5% β-mercaptoethanol and at 40°C in 0.5X SSC/0.5% β-mercaptoethanol, treated with RNAses A (20 μg/ml) and T_1 (50 U/ml), washed again for 2 hours at 45°C in 2X SSC/50% formamide/0.5% β-mercaptoethanol. After three washes in 0.1X SSC at room temperature, the slides were dehydrated.

Autoradiography was performed (Kodak NTB-2 nuclear track emulsion) for 4 to 20 days, and sections were counterstained in 5% Giemsa dye.

Control Experiments

The specificity of the cDNA probes was ascertained by computerized homology search and *in situ* hybridization experiments in the fetal growth plate. Four samples of fetal growth plate cartilage were investigated as controls in parallel and showed the typical matrix gene expression pattern[4,5,37]: moderate to high mRNA expression levels for collagen types II, IX, and XI as well as aggrecan and link protein in chondrocytes of all zones (resting, proliferative, hypertrophic) of the fetal growth plate cartilage (see Figure 2, a, d, g, m, p, and s). Type X collagen mRNA expression was restricted to hypertrophic zone chondrocytes (see Figure 2j). No significant amounts of type I or III collagen mRNA expression were found in any fetal chondrocytes except very weak expression levels in two samples at the very surface (epichondral area; not shown). Non-neoplastic tissue within the tumor specimens served as internal positive and negative controls (see Results). Sense transcripts were used as nonspecific negative controls and never showed more than background signals (not shown). A probe for 18 S rRNA[36] was used as a positive control for preservation of RNA during the technical procedure and showed strong signal in the vast majority of cells of all samples analyzed (not shown). Areas containing significant foci of cell necrosis were not used for this study.

As negative control for immunohistochemical staining, the primary antibody was replaced either by preimmune serum, nonspecific mouse or rabbit serum (BioGenex, San Ramon, CA), or Tris-buffered saline (pH 7.2).

Results

To account for the morphological heterogeneity between and within different specimens, tumors were subdivided into chondroid, chondromyxoid, myxoid, and anaplastic areas (Figure 1; for details on morphology see Refs. 38 and 39). Chondromas and well differentiated chondrosarcomas (low grade, G1) showed mostly lobular chondroid and chondromyxoid areas, whereas poorly differentiated chondrosarcomas (high grade, G3) consisted mostly of myxoid and anaplastic tissue. Moderately differentiated chondrosarcomas (G2) showed chondroid, chondromyxoid, and myxoid tissue types in various proportions.

Neoplastic cells in all except the anaplastic tumor areas were positive for S-100 protein (Figure 1, g–i), which distinguished the tumor cells from non-neoplastic cells such as fibroblasts and osteoblasts.

In chondroid tumor areas, the single or clustered neoplastic cells had a normochromatic nucleus and a round shape and were surrounded by an abundant, hyaline extracellular matrix (Figure 1, a and d), which was strongly positive for proteoglycans (Figure 1a) and collagen type II (Figure 1d). Many of the neoplastic cells expressed high mRNA levels for collagen types II (Figure 2, b and e), IX (Figure 2h), and XI (Figure 2n), aggrecan core (Figure 2q), and link protein (Figure 2t), which was comparable to fetal growth plate chondrocytes (Figure 2, a, d, g, m, p, and s). It is noteworthy that morphologically identical cells within the same tumors did not show any mRNA expression for these proteins (not shown), although histochemical and immunohistochemical analysis showed the same distribution of matrix components in these sites as in areas of actively synthesizing cells. This indicated previous synthetic activity of these cells, which were now silent. Most of these cells were not necrotic as shown by *in situ* hybridization with the probe for 18 S rRNA.

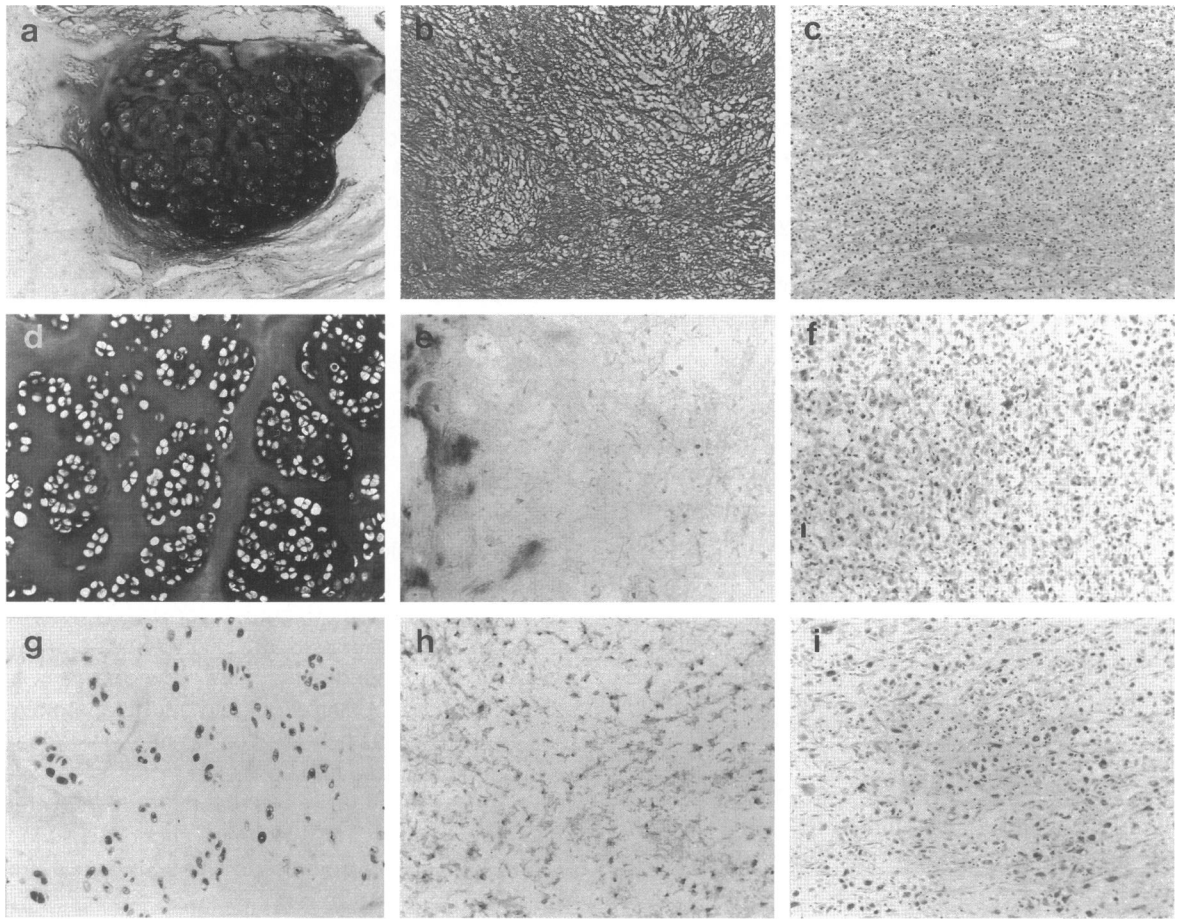

Figure 1. *Low-power micrographs showing the morphological appearance and proteoglycan* (a *to* c; *toluidine blue staining*) *and collagen type II* (d *to* f) *distribution in chondroid* (a, d, *and* g), *myxoid* (b, e, *and* h), *and anaplastic* (c, f, i) *tumor areas.* g *to* i: *High-power micrographs showing the expression of S-100 protein in these areas. In anaplastic areas, only nuclear counterstain but no immunohistochemical signal was observed for S-100 protein. Magnification,* ×*50* (a *to* c) *and* ×*100* (d *to* i).

In nearly all chondrosarcomas and in three of the chondromas, there was focal expression of collagen type X mRNA and protein (Figures 2k and 3, a and b). In two chondrosarcomas, neoplastic chondrocytes also expressed type I collagen (Figure 3, c and d). No significant amount of type III collagen mRNA or protein was observed in these or other cells of chondroid areas.

Cells in chondromyxoid tumor areas (not shown) expressed the typical cartilage matrix components as in chondroid areas. The histochemical and immunohistochemical staining for proteoglycans and collagen type II was present but irregular. Additionally, type I and, to a lesser extent, type III collagen appeared in many sites in the extracellular matrix. The cells of these areas therefore showed an intermediate cellular phenotype.

In myxoid tumor areas (Figure 1b), the cells were spindle shaped or stellate and surrounded by a less abundant extracellular matrix. Histochemical analysis showed a considerably lower content of collagens and proteoglycans (Figures 1b and 4f) in these areas. Immunohistochemically, a severe reduction or absence of type II collagen was found (Figures 1e and 4c). The lack of cartilage matrix gene expression was clearly confirmed at the mRNA level; the neoplastic cells expressed little, if any, collagen types II, IX, X, and XI or aggrecan and link protein mRNAs (Figure 2, c, f, i, l, o, r, and u). Instead, expression of collagen types I (Figure 4, a and b) and III (Figure 4, d and e) was found at the protein and mRNA level. Some cells were negative for all mRNAs (not shown), although *in situ* hybridization with the 18 S rRNA probe showed that these cells were not necrotic.

In anaplastic areas of high-grade neoplasms, the neoplastic cells were pleomorphic with polymorphic, mostly hyperchromatic nuclei (Figure 1, f and i). The extracellular matrix was sparse, consisting of collagen types I and III and presumably associated with numerous vascular structures

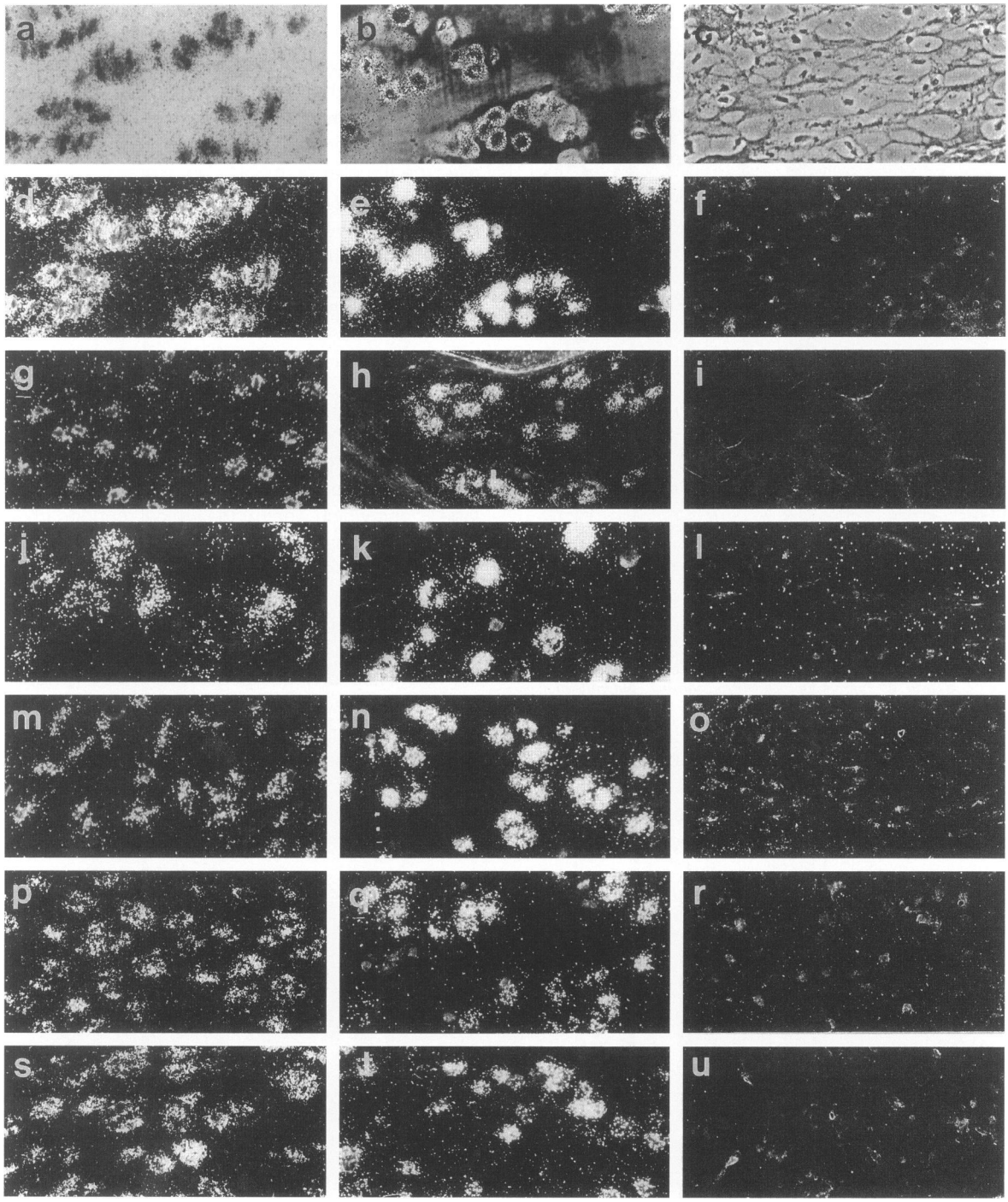

Figure 2. In situ *hybridization analysis of chondroid* (**b**, **e**, **h**, **k**, **n**, **q**, *and* **t**) *and myxoid* (**c**, **f**, **i**, **l**, **o**, **r**, *and* **u**) *areas in chondrosarcomas in comparison with fetal growth plate cartilage* (**a**, **d**, **g**, **j**, **m**, **p**, *and* **s**) *using riboprobes for* α*1(II)* (**a** *to* **f**), α*1(IX)* (**g** *to* **i**), α*1(X)* (**j** *to* **l**), α*1(XI)* (**m** *to* **o**), *aggrecan core* (**p** *to* **r**), *and link protein* (**s** *to* **u**). **a** *to* **c**: *Contrast phase photographs (corresponding to* **d** *to* **f**). **d** *to* **u**: *Dark-field photographs. Magnification,* ×*300.*

found between the tumor cells. No collagen types II and X or proteoglycan (Figure 1, c and f) was detectable. These neoplastic cells were negative for S-100 protein (Figure 1i).

Non-Neoplastic Tissue (Not Shown)

Non-neoplastic extracellular matrix and cells showed a typical mRNA expression and protein distribution

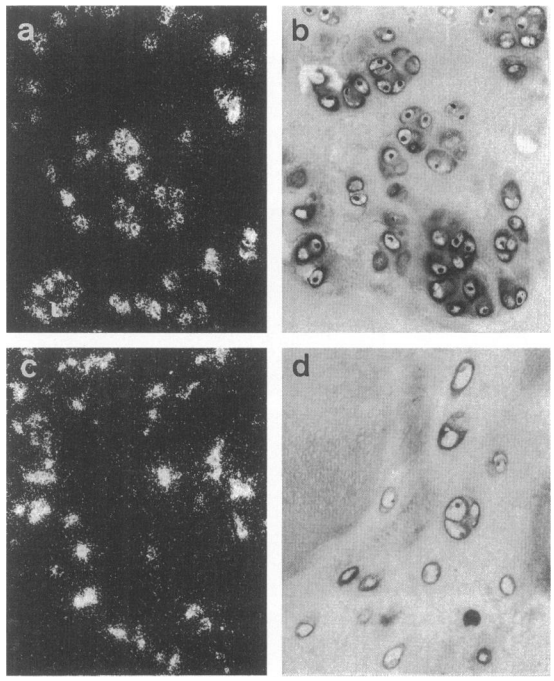

Figure 3. *In morphologically chondroid neoplastic areas, focally strong expression of collagen type X was observed at the mRNA* (a) *and protein* (b) *level. In some of these areas, expression of type I collagen mRNA* (c) *and protein* (d) *was also detected.* a *and* c: In situ *hybridization with probes for* α*1(X)* (a) *and* α*1(I)* (c)*; dark-field photographs.* b *and* d: *Immunohistochemical staining. Magnification,* ×*250.*

pattern.[4,40,41] The fibrous bands separating the tumor lobules were positive for collagen types I and III. The matrix of surrounding bone was solely positive for collagen type I. The endosteal layer was additionally positive for collagen type III. Type I collagen mRNA was the only gene found to be strongly expressed by osteoblasts lining bone trabecules around and in between the tumor lobules. Mesenchymal bone marrow cells as well as fibroblastic cells in the interlobular septae expressed additionally type III collagen mRNA. No expression of collagen types II, IX, X, and XI or aggrecan core and link protein mRNAs was seen in the non-neoplastic fibrous or osseous tissue.

All non-neoplastic cells were negative for S-100 protein in the specimens investigated.

Discussion

The hallmark of all differentiated cartilaginous neoplasms was the occurrence of neoplastic cells, which showed the same gene expression profile as mature chondrocytes of the fetal growth plate cartilage[4,5] and which were of the typical rounded chondrocytic cell shape. These mature neoplastic chondrocytes were responsible for the formation of the hyaline-cartilage-like extracellular matrix, which is highly characteristic for these neoplasms.[24] Our results confirm and extend earlier biochemical and immunohistochemical reports,[24,27,42] which showed that the matrix composition of cartilaginous tumors is qualitatively similar to normal hyaline cartilage. However, they do not support the notion of Mankin and co-workers[24,25] that the collagen content is much lower than in hyaline cartilage irrespective of the local matrix composition. Our histochemical and im-

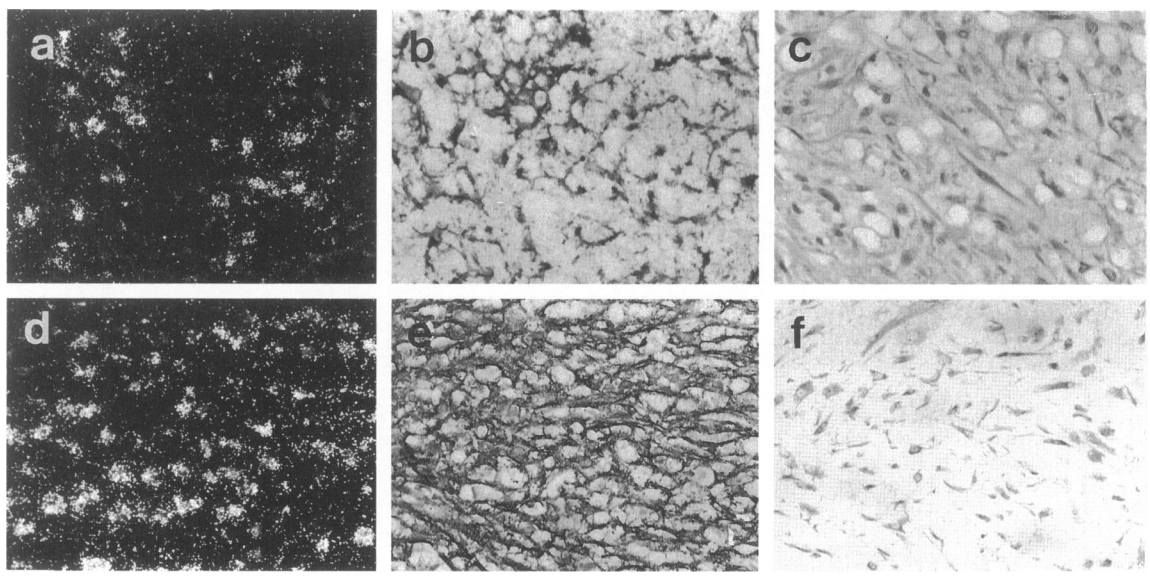

Figure 4. *In morphologically myxoid tumor areas, collagen types I* (b) *and III* (e)*, but not collagen type II* (c)*, and reduced proteoglycans* (f) *were detectable.* In situ *hybridization confirmed the expression of type I* (a) *and III* (d) *collagen by the spindle-shaped or stellate neoplastic cells at the mRNA level.* a *and* d: In situ *hybridization analysis; dark-field photographs.* b, c, *and* e: *Immunohistochemical staining.* f: *Toluidine blue staining. Magnification,* ×*350.*

munohistochemical data showed that chondroid areas had a high content of cartilaginous collagens (types II, IX, X, and XI). The extracellular matrix of myxoid areas, on the other hand, had a much lower content of collagens[43,44] and they were composed of noncartilaginous types (I and III).

Using collagen gene expression pattern as a well established tool to monitor chondrocyte differentiation,[1,2] our study demonstrates that neoplastic chondrocytes *in vivo* do show the full differentiation capacity of their physiological counterparts. Besides mature chondrocytes, hypertrophic differentiation with the expression of collagen type X was observed. The onset of type I collagen expression without type III collagen in differentiated, rounded neoplastic chondrocytes of chondroid areas represents the first experimental evidence of the potential of mammalian chondrocytes to undergo differentiation to osteoblast-like cells *in vivo*. However, this needs further investigation. In some nonchondroid areas, the cells were spindle shaped and expressed collagen types I and III instead of the cartilage typical genes, features that are characteristic for so-called dedifferentiated chondrocytes *in vitro*.[16,17,19] Interestingly, S-100 protein was positive in these neoplastic cells. S-100 protein is a specific marker for chondrocytic mesenchymal cells[45] and thus distinguishes these cells, despite many phenotypic similarities, from fibroblasts and other cell types.

All of the observed phenotypes, including the dedifferentiated phenotype, were forms of differentiated neoplastic cells in contrast to the undifferentiated cells found in the anaplastic areas of poorly differentiated neoplasms, which were negative for all markers analyzed for the chondrocytic phenotype including S-100 protein. Thus, our data show that the term dedifferentiated, although well established in the literature,[46–49] is a misnomer.

It remains unclear from our study whether the observed differentiation patterns are early, subsequently stable events during tumor development or whether phenotypic switches do occur more frequently during the growth of the neoplasms. From our studies, it is also unclear whether neoplastic cell differentiation is reversible within the neoplasms, as happens during chondrocyte dedifferentiation *in vitro*.[47,50] Our study also does not allow significant conclusions on the cellular origin of these neoplasms, in particular, whether any sort of primary tumor cell exists that resembles fetal chondroprogenitor cells and expresses the specific splice product COL2A.[3,4] Most likely, the origin of these tumors are multipotent mesenchymal stem cells, which were demonstrated to exist in virtually all connective tissues of the body (for review see Ref. 51).

The striking heterogeneity of the extracellular matrix appearance not only between different but also within chondroid tumors is one of the characteristic and poorly understood features of these neoplasms.[24,25] Our results do not support the assumption of Mankin and co-workers that the heterogeneity of the extracellular tumor matrix is a phenomenon primarily due to different cell clones with different genetic errors.[24,25] Instead, the phenotypic diversity of the neoplastic cells combined with a varying cellular synthetic activity results in a rather large diversity of biochemical composition and abundance of the tumor matrix and cell and tissue morphology. In support of this conclusion, in our study, matrix morphology correlated well with matrix gene expression patterns. However, comparison of the cellular DNA content with tumor appearance suggested a correlation with the distribution pattern of the cellular phenotypes. Thus, the mature phenotype was mainly found in well differentiated neoplasms with normally appearing nuclei, whereas the anaplastic cell type was restricted to the poorly differentiated neoplasms with highly pleomorphic and hyperchromatic aneuploid nuclei.

Our data also substantiate why matrix appearance is one of the criteria of defining the tumor grade[28] and, thus, one predictor for biological and clinical behavior of the neoplasms. The dedifferentiated cellular phenotype, which *in vitro* shows significant proliferative activity,[52] is the dominant phenotype in chondrosarcomas with intermediate growth rates.[28] The anaplastic cells, which were restricted to poorly differentiated neoplasms, exhibit a high proliferation and growth rate.[53] In contrast, the mature phenotype, which is responsible for the hyaline-cartilage-like matrix of the tumor, is the predominant phenotype in chondromas and well differentiated chondrosarcomas. Both mature chondrocytes *in vitro* as well as well differentiated chondrogenic neoplasms *in vivo* show little proliferative activity,[52,53] which is reflected by the low cell content in chondroid tumor areas and the slow growth of these neoplasms. Thus, our study provides good experimental evidence that phenotypic features correlate not only with tumor matrix biochemistry and morphology but also with growth and clinical behavior of the neoplasms. Furthermore, malignant neoplasms showed greater phenotypic diversity compared with the benign chondromas. However, our results do not provide specific biochemical or molecular markers for malignancy or grading of cartilaginous tumors.[24] This is particularly true as many expression patterns were found focally within the neoplasms. Whether

the appearance of certain collagen types might be of independent diagnostic and prognostic value needs additional investigation.[26,27]

Overall, we were able to show true chondrocytic differentiation and phenotypic modulation of neoplastic chondrocytes *in vivo*. We provide evidence for two phenotypes for the first time in mammalian chondrocytes *in vivo*, which were so far only described *in vitro* or in the chick system. Despite the considerable heterogeneity and many transitional areas within the tumors, we were able to establish a relationship between neoplastic cellular phenotype, tumor matrix biochemistry, and morphological appearance within the chondrogenic tumors.

Acknowledgments

We are grateful to Dr. E. Vuorio, University of Turku, Finland, and Dr. B. Olsen, Harvard University, Boston, MA, for generously providing us with cDNA probes for α_1(II), α_1(III), and α1(IX) collagens. We thank Ms. S. Blank and Ms. C. Winkelman for expert technical assistance and Ms. G. Herbig for expert photographic help.

References

1. von der Mark K: Differentiation, modulation, and dedifferentiation of chondrocytes. Rheumatology 1986, 10: 272–315
2. Cancedda R, Descalzi-Cancedda F, Castagnola P: Chondrocyte differentiation. Int Rev Cytol 1995, 159: 265–358
3. Sandell LJ, Morris NP, Robbins JR, Goldring MB: Alternatively spliced type II procollagen mRNAs define distinct populations of cells during vertebral development: differential expression of the amino-propeptide. J Cell Biol 1991, 114:1307–1319
4. Vornehm SI, Dudhia J, von der Mark K, Aigner T: Expression of collagen types IX and XI as well as other major cartilage matrix components by human fetal chondrocytes *in vivo*. Matrix Biol 1996, 15:91–98
5. Sandberg M, Vuorio E: Localization of types I, II, and III collagen mRNAs in developing human skeletal tissues by *in situ* hybridization. J Cell Biol 1987, 104:1077–1084
6. Müller PK, Lemmen C, Gay SW, Gauss V, Kühn K: Immunochemical and biochemical study of collagen synthesis by chondrocytes in culture. Exp Cell Res 1977, 108:47–55
7. Mayne R, Brewton RG, Mayne PM, Baker JR: Isolation and characterization of the chains of type V/type XI collagen present in bovine vitreous. J Biol Chem 1993, 268:9381–9386
8. Brewton RG, Wright DW, Mayne R: Structural and functional comparison of type IX collagen-proteoglycan from chicken cartilage and vitreous humor. J Biol Chem 1991, 266–8:4752–4757
9. Reichenberger E, Aigner T, von der Mark K, Stöss H, Bertling W: *In situ* hybridization studies on the expression of type X collagen in fetal human cartilage. Dev Biol 1991, 148:562–572
10. Schmid TM, Linsenmayer TF: Immunohistochemical localization of short chain cartilage collagen (type X) in avian tissues. J Cell Biol 1985, 100:598–605
11. Hiltunen A, Aro HT, Vuorio E: Regulation of extracellular matrix genes during fracture healing in mice. Clin Orthop Rel Res 1993, 297:23–27
12. Aigner T, Dietz U, Stöss H, von der Mark K: Differential expression of collagen types I, II, III, and X in human osteophytes. Lab Invest 1995, 73:236–243
13. Cancedda FD, Gentili C, Manduca P, Cancedda R: Hypertrophic chondrocytes undergo further differentiation in culture. J Cell Biol 1992, 117:427–435
14. Kirsch T, Swoboda B, von der Mark K: Ascorbate independent differentiation of human chondrocytes *in vitro*: simultaneous expression of type I and X collagens and matrix mineralization. Differentiation 1992, 52:89–100
15. Roach HI, Erenpreisa J, Aigner T: Osteogenic differentiation of hypertrophic chondrocytes involves asymmetric cell divisions and apoptosis. J Cell Biol 1995, 131:483–494
16. Benya PD, Padilla SR, Nimni ME: Independent regulation of collagen types by chondrocytes during the loss of differentiated function in culture. Cell 1978, 15:1313–1321
17. Benya PD, Padilla SR, Nimni ME: The progeny of rabbit articular chondrocytes synthesize collagen types I and III and type I trimer, but not type II: verifications by cyanogen bromide peptide analysis. Biochemistry 1977, 16–5:865–872
18. von der Mark K, Gauss V, von der Mark H, Müller PK: Relationship between cell shape and type of collagen synthesized as chondrocytes lose their cartilage phenotype in culture. Nature 1977, 267:531–532
19. Quarto R, Dozin B, Bonaldo P, Cancedda R, Colombatti A: Type VI collagen expression is upregulated in the early events of chondrocyte differentiation. Development 1993, 117:245–251
20. Goldring MB, Birkhead JR, Suen L-F, Yamin R, Mizuno S, Glowacki J, Arbiser JL, Apperley JF: Interleukin-1-modulated gene expression in immortalized human chondrocytes. J Clin Invest 1994, 94:2307–2316
21. Finer MH, Gerstenfeld LC, Young D, Doty P, Boedtker H: Collagen expression in embryonic chicken chondrocytes treated with phorbol myristate acetate. Mol Cell Biol 1985, 5:1415–1424
22. Yoshimura M, Jiminez SA, Kaji A: Effects of viral transformation on synthesis and secretion of collagen and fibronectin-like molecules by embryonic chick chondrocytes in culture. J Biol Chem 1981, 256:9111–9117
23. Kolettas E, Buluwela L, Bayliss MT, Muir HI: Expression of cartilage-specific molecules is retained on long-

term culture of human articular chondrocytes. J Cell Sci 1995, 108:1991–1999
24. Mankin HJ, Cantley KP, Lippiello L, Schiller AL, Campbell CJ: The biology of human chondrosarcoma. I. Description of the cases, grading, and biochemical analysis. J Bone Joint Surg 1980, 62-A:160–175
25. Mankin HJ, Cantley KP, Schiller AL, Lippiello L: The biology of human chondrosarcoma. II. Variation in chemical composition among types and subtypes of benign and malignant cartilage tumors. J Bone Joint Surg 1980, 62-A:176–188
26. Remberger K, Gay SW: Immunohistochemical demonstration of different collagen types in the normal epiphyseal plate and in benign and malignant tumors of the bone. Zeitung Krebsforschung 1977, 90:95–106
27. Ueda Y, Oda Y, Tsuchiya H, Tomita K, Nakanishi I: Immunohistological study on collagenous proteins of benign and malignant human cartilaginous tumours of bone. Virchows Arch A 1990, 417:291–297
28. Evans HL, Ayala AG, Romsdahl MM: Prognostic factors in chondrosarcoma of bone. Cancer 1977, 40:818–831
29. Rosenberg LC: Chemical basis for the histological use of safranin O in the study of articular cartilage. J Bone Joint Surg 1971, 53-A:69–82
30. von der Mark K, Kirsch T, Nerlich AG, Kub A, Weseloh G, Glückert K, Stöb H: Type X collagen synthesis in human osteoarthritic cartilage. Arthritis Rheum 1992, 35:806–811
31. Klareskog L, Johnell O, Hulth, Holmdahl R, Rubin K: Reactivity of monoclonal anti type II collagen antibodies with cartilage and synovial tissue in rheumatoid arthritis and osteoarthritis. Arthritis Rheum 1986, 29: 730–738
32. Girkontaité I, Frischholz S, Lammi P, Wagner K, Swoboda B, Aigner T, von der Mark K: Immunolocalization of type X collagen in normal fetal and adult osteoarthritic cartilage with monoclonal antibodies. Matrix Biol 1996, 15:231–238
33. Aigner T, Bertling W, Stöss H, Weseloh G, von der Mark K: Independent expression of fibril-forming collagens I, II, and III in chondrocytes in human osteoarthritic cartilage. J Clin Invest 1993, 91:829–837
34. Dudhia J, Davidson CM, Wells T, Vynos D, Hardingham TE, Bayliss MT: Age-related studies in the content of the carboxyl-terminal region of aggrecan in human cartilage. Biochem J 1996, 313:933–940
35. Dudhia J, Hardingham TE: The primary structure of human cartilage link protein. Nucleic Acids Res 1990, 18:1292
36. Aigner T, Stöss H, Weseloh G, Zeiler G, von der Mark K: Activation of collagen type II expression in osteoarthritic and rheumatoid cartilage. Virchows Arch B 1992, 62:337–345
37. Mundlos S, Meyer R, Yamada Y, Zabel B: Distribution of cartilage proteoglycan (aggrecan) core protein and link protein gene expression during human skeletal development. Matrix 1991, 11:339–346
38. Unni KK: Dahlin's bone tumors, ed 5. Philadelphia, Lippincott-Raven, 1996, pp 1–463
39. Schajowicz F: Tumors and tumorlike lesions of bone, ed 2. Berlin, Springer-Verlag, 1994
40. Carter DH, Sloan P, Aaron JE: Immunolocalization of collagen types I and III, tenascin, and fibronectin in intramembranous bone. J Histochem Cytochem 1991, 39:599–606
41. Sandberg M, Autio-Harmainen H, Vuorio E: Localization of the expression of types I, III, and IV collagen, TGF-β and c-*fos* genes in developing human calvarial bones. Dev Biol 1988, 130:324–334
42. Kawahima A, Ueda Y, Tsuchiya H, Tomita K, Nagai Y, Nakanishi I: Immunohistochemical localization of collagenous proteins in cartilaginous tumors: characteristic distribution of type IX collagen. J Cancer Res Clin Oncol 1993, 120:35–40
43. Povsil C, Matejovsky Z: A comparative ultrastructural study of chondrosarcoma, chondroid sarcoma, chordoma, and chordoma periphericum. Pathol Res Pract 1985, 179:546–559
44. Fu Y-S, Kay S: A comparative ultrastrucutural study of mesenchymal chondrosarcoma and myxoid chondrosarcoma. Cancer 1974, 33:1531–1542
45. Okajima K, Honda I, Kitagawa T: Immunohistochemical distribution of S-100 protein in tumors and tumor-like lesions of bone and cartilage. Cancer 1988, 61:792–799
46. Holtzer H, Abbott J, Lash JW, Holtzer S: The loss of phenotypic traits by differentiated cells *in vitro*. I. Dedifferentiation of cartilage cells. Proc Natl Acad Sci USA 1960, 46:1533–1542
47. Benya PD, Shaffer JD: Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. Cell 1982, 30:215–224
48. Bonaventure J, Khadom N, Cohen-Solal L, Ng KH, Bourguignon J, Lasselin C, Freisinger P: Re-expression of cartilage-specific genes by dedifferentiated human articular chondrocytes cultured in alginate beads. Exp Cell Res 1994, 212:97–104
49. Harrison ET, Luyten FP, Reddi AH: Osteogenin promotes reexpression of cartilage phenotype by dedifferentiated articular chondrocytes in serum-free medium. Exp Cell Res 1991, 192:340–345
50. Benya PD, Brown PD, Padilla SR: Microfilament modification by dihydrocytochalasin B causes retinoic acid-modulated chondrocytes to reexpress the differentiated collagen phenotype without a change in shape. J Cell Biol 1988, 106:161–170
51. Caplan AI: Mesenchymal stem cells. J Orthop Res 1991, 9:641–650
52. Ronot X, Hecquet C, Jaffray P, Guiguet M, Adolphe M, Fontagne J, Lechat P: Proliferation kinetics of rabbit articular chondrocytes in primary culture and at the first passage. Cell Tissue Kinetics 1983, 16:531–537
53. Hasegawa T, Seki K, Yang P, Mirose T, Mizawa K, Wada T, Wakayashi J-I: Differentiation and proliferative activity in benign and malignant cartilage tumors of bone. Hum Pathol 1995, 26:838–845