LETTERS

Pigment epithelium derived factor—the product of the EPC-1 gene—is expressed by articular chondrocytes and up regulated in osteoarthritis

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Proper function of a loadbearing joint depends on the structural integrity of articular cartilage, which can absorb and respond to mechanical stress.¹ Increased synthesis of VEGF-A is assumed to be involved in cartilage neovascularisation in osteoarthritis (OA), thus contributing to cartilage damage during the course of OA.^{2,3}

Previously, we and others have provided experimental evidence that VEGF-A synthesis by OA chondrocytes is controlled by the transcription factor hypoxia-inducible factor-1 α .^{2–4} In contrast, pigment epithelium derived factor (PEDF) is one of the most potent natural inhibitors of angiogenesis and was recently described as being present in growth plate cartilage.⁵ PEDF constitutes a 50 kDa protein and is encoded by the EPC-1 gene.^{6,7} Based on our previous work, we aimed to determine whether PEDF is expressed in human normal and OA knee cartilage.

Twenty nine cartilage samples were obtained from patients during total knee replacement. In addition, nine human normal cartilage samples were obtained during necropsies. After fixation, specimens were embedded in paraffin wax, stained with safranin O, and were graded according to Mankin *et al.*⁸ For immunodetection of PEDF two different antibodies were used (polyclonal 1:100, Santa-Cruz Biotechnology Inc, USA; monoclonal 1:200, Chemicon International Inc, USA). Immunoreaction was visualised using streptavidin and biotin labelled with alkaline phosphatase. Positive chondrocytes were counted under a high power field.

For conventional and real time polymerase chain reaction (PCR) total RNA was isolated from nine normal and eight OA cartilage samples as described.⁹ After reverse transcription 20 ng cDNA were amplified through 35 cycles in all experiments and analysed on 1.5% agarose gels. The following primer sequences were used: glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward 5'-CGGAGTCAACGGATTGGTCTGAT-3', reverse 5'-AGCCTTCTCCATG-GTVGGTGAAGAC-3' (product 306 bp)²; PEDF forward 5'-TATGACTTGATCAGCAG-3', reverse 5'-AGCTTCATCTCCTGCAGGGA-3' (product 675 bp). Quantitative real time polymerase chain reaction (RT-PCR) was performed using the ABI Prism 7900 sequence detection system (Applied Biosystems, Foster City, USA) and QuantiTect Probe RT-PCR Kit (Qiagen) for one-step RT-PCR. Relative quantification was calculated using the standard curve method (normalised to GAPDH). A primer/probe set for GAPDH was used as described.⁹ The primer/probe set for PEDF (TaqMan Assays-on-Demand Gene Expression Product) was purchased from Applied Biosystems.

In human normal cartilage, PEDF stained chondrocytes were detectable in the superficial zone and scattered throughout all cartilage layers (fig 1A (a–c)). In OA cartilage, the number of PEDF positive chondrocytes increased in

parallel with progressive cartilage damage (fig 1A (d–i) and 1B). Identical immunostaining results were obtained with both antibodies against PEDF (fig 1A (h, i)). Expression of PEDF in vivo was confirmed by conventional PCR (fig 1C). Determination of PEDF transcripts by real time PCR confirmed our semiquantitative immunohistology results. PEDF mRNA levels were ~5.3-fold increased in RNA extracts from OA samples in comparison with extracts from healthy cartilage (fig 1D).

As far as we know, this is the first study describing gene and protein expression of PEDF in human normal and OA cartilage by means of immunohistochemistry, conventional and real time PCR. The anti-angiogenic properties of this 50 kDa molecule are of special interest given the avascular structure of healthy articular cartilage. In contrast, the pathogenesis of OA is characterised by scattered invasion of blood vessels from the subchondral bone into hyaline cartilage. It is widely assumed that this phenomenon might result from increased synthesis of proangiogenic factors—for example, VEGF-A.¹⁰ However, in this study we could demonstrate a significant up regulation of PEDF, a strong anti-angiogenic factor, in OA compared with normal cartilage. We therefore conclude that PEDF acts as an anti-angiogenic factor limiting the driving force of proangiogenic molecules and ultimately controlling blood vessel invasion into hyaline cartilage during the course of OA.

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Figure 1 Evidence for an increased expression of PEDF during osteoarthritis. (A) Sections of normal and OA cartilage stained with safranin O and immunostained for PEDF are demonstrated. (a) Normal articular cartilage (Mankin-1) with a smooth surface and a high content of proteoglycans (safranin O staining); (b) immunostaining for PEDF displayed single weak stained chondrocytes (arrows) in the middle zone (same specimen as that shown in (a)). (c) no immunostaining for PEDF were seen in the deep zone of this cartilage specimen; (d) moderate OA lesion (Mankin-5) with a decreased proteoglycan staining of the upper cartilage (safranin O staining); (e) immunohistochemical analysis of PEDF in an adjacent section showing an increased number of PEDF positive chondrocytes in the upper areas and within the deep zone; (PEDF stained chondrocytes are indicated by arrows); (f) PEDF chondrocytes in the deep zone of OA cartilage; (g) severe OA lesion (Mankin-11) with deep clefts, chondrocyte clusters, and a strong reduction of proteoglycans (safranin O staining); (h) immunohistochemical analysis of PEDF in the same section as that shown in (g) (note the strong immunostaining of clustered chondrocytes as indicated by the arrows); (i) severe OA cartilage specimen immunostained with a monoclonal antibody against PEDF, showing the same cellular and pericellular staining pattern (two negative chondrocytes are indicated by arrows). Bar in (a) represents 300 μ m in a, d, 180 μ m in e, g-i, and 60 μ m in b, c, f. (B) Scatterplots of immunopositive chondrocytes (percentages) as a function of Mankin's grade. Blue circles, counted positive chondrocytes stained with the polyclonal antibody used; black triangles, counted positive chondrocytes stained with the monoclonal antibody used. (C) Demonstrated are products of conventional PCR (35 cycles) for PEDF (675 bp) compared with GAPDH (306 bp) analysed on 1.5% agarose gels containing ethidium bromide. PCR products of normal (n=6) versus OA (n=7) cartilage samples are shown. (D) The result of real time PCR analysis for PEDF transcripts is shown. Bars represent the several-fold changes of PEDF mRNA levels (normalised to GAPDH) in OA (n=8) compared with healthy cartilage samples (n=9). Bars represent means (SD). * $p < 0.05$, ** $p < 0.01$ v healthy samples.

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Personal attributes as determinants of timely care in rheumatoid arthritis

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Recently, early intensive treatment has become important in the treatment of rheumatoid arthritis (RA). Evidence from clinical trials and from patient cohorts in daily practice has shown that early disease modifying antirheumatic drug treatment is mandatory to avoid damage and loss of function.¹ Therefore, patients with suspicion of RA should be referred to a rheumatologist as soon as possible.

This has led to the development of “early arthritis clinics”.² However, a diagnosis of early RA is difficult for general practitioners (GPs),³ sometimes leading to inappropriate referrals. A solution may be joint consultations of GPs and rheumatologists⁴ or specific training of GPs.

A few years ago, the Patient Partner Program (PPP) was established in Belgium. The PPP is an educational programme in which trained patients with RA teach medical students and general practitioners how to diagnose and evaluate RA.⁵ A direct objective is to improve musculoskeletal examination skills of physicians, leading to appropriate early referrals.

Other hurdles for timely care appear to be patient related. Women are often referred later than men⁶; however, it is not clear if this is due to sex alone, gender behaviour or other interfering factors.

Patient partners are selected by their treating rheumatologist based on communication skills, motivation, and positive attitude towards their disease and learning capacities. Such characteristics might also influence their behaviour of seeking health care.

As part of an evaluation of the PPP in Belgium, we assessed the association of being a patient partner with disease characteristics (medical records), disability (Health Assessment Questionnaire (HAQ)), perceived health status (Dutch AIMS⁷), and coping (UCL⁸). Twenty one patient partners were compared with 28 randomly selected patients with RA who were not patient partners. All were receiving regular ambulatory care.

The patient partner group had a significantly longer disease duration, but age and sex distribution were similar (table 1). We found that patient partners had more social activities ($p = 0.013$), had a better ability to work ($p = 0.043$), had a lower stress level ($p = 0.002$), and had a more positive mood ($p = 0.016$). Patient partners also demonstrated more active coping strategies ($p = 0.026$), more reassuring thoughts ($p = 0.040$), and less depressive reactions ($p = 0.001$). Interestingly enough, we observed that the time between onset of symptoms and the diagnosis of RA was significantly shorter in the patient partner group than in the control group.

Although the differences in health status and coping are logically explained by the selection criteria for becoming a patient partner, the personal attributes of these patients apparently resulted in an earlier diagnosis of RA, even when they were not yet trained as patient partners. We feel that these findings add to the understanding of determinants of timely care in patients with RA, such as coping and personality styles. Investigations of the influence of gender

Table 1 Patient characteristics

Characteristics	Patient partners (n = 21)	Control group (n = 28)	p Value
Sex (% female)	95	98	
Mean age (years)	45.3	47.9	0.424†
Mean disease duration (years)	15.7	9.3	<0.01‡
Mean time between complaints and diagnosis (years)	0.8	2.7	0.001†
Surgical intervention for RA (%)	71	57	0.305*
Disability score (mean HAQ)	1.4	1.2	0.321†

* χ^2 Test; †Mann-Whitney U test; ‡t test.