shown in the Table. One near-optimal model contains age plus three MMs (COMP, 846, and TNF type II receptor), and places approximately 90% of both OAs and the normals into the correct diagnosis. The simplest effective model is the one containing age plus TNF type II receptor, which places 90% of normals and 86% of OA patients into the correct diagnostic category.

Conclusion

Based on the analysis of 14 MMs, reliable discrimination of knee OA with better than 90% discrimination of OA and normals could be obtained. It is interesting that the best discrimination in this analysis came from the use of one marker from each of three groups: the inflammatory markers (TNF type II receptor), markers of cartilage synthesis (846) and markers of cartilage turnover (COMP).

References

Eyre D, Koob T, Van Ness K. Quantitation of hydroxypyridinium crosslinks in collagen by high-performance liquid chromatography. Analytical Biochem 1984; 137: 380–8.

Lohmander L S, Lark M W, Dahlberg L, Walakovits L A, Roos H. Cartilage matrix metabolism in osteoarthritis: Markers in synovial fluid, serum, and urine. Clin Biochem 1992; 25: 167–74.

Månsson B, Carey D, Alini M, Ionescu M, Rosenberg L C, Poole A R, Heinegård D, Saxne T. Cartilage and bone metabolism in rheumatoid arthritis. Differences between rapid and slow progression of disease identified by serum markers of cartilage metabolism. J Clin Invest 1995; 95: 1071–7.

Poole A R, Ionescu M, Swan A, Dieppe P. Changes in cartilage metabolism in arthritis are rellected by altered serum and synovial fluid levels of glycosaminoglycan epitopes on fragments of the cartilage proteoglycan aggrecan: Implications for pathogenesis. J Clin Invest 1994; 94: 25–33.

Poole A R, Webber C, Reiner A, Roughley P J. Studies of a monoclonal antibody to skeletal keratan sulfate. Importance of antibody valency. Biochem J 1989; 260: 849–56.

Saxne T, Heinegård D. Cartilage oligomeric matrix protein: a novel marker of cartilage turnover detectable in synovial fluid and blood. Br J Rheumatol 1992; 31: 583–91.

Saxne T, Zunino L, Heinegård D. Increased release of bone sialoprotein into synovial fluid reflects tissue destruction in rheumatoid arthritis. Arthritis Rheum 1995; 38: 82–90.

Evolution of a matrix metalloproteinase assay to assess inhibitor potency and selectivity

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Matrix metalloproteinases (MMPs) are a class of zinc-containing extracellular proteinases involved in extracellular matrix turnover and remodeling. As such, these enzymes are critical in maintaining the proper biochemical composition and physical characteristics of all tissues, but in particular those tissues whose composition is largely extracellular matrix, such as cartilage. Under normal physiological conditions, constitutive expression of the MMPs is low, as is their catalytic activity which is held in check by naturally occurring inhibitors termed TIMPs (tissue inhibitor of metalloproteinases). However, under pathologic conditions such as rheumatoid and osteoarthritis, MMP expression in cartilage is disregulated. MMP levels are high with enzymic activity exceeding the level of the natural inhibitors. This condition leads to a loss of proteoglycan and collagen from articular cartilage culminating in the ultimate destruction which characterizes the pathology of arthritic diseases. Based upon this scenario, intensive efforts are focused on the identification of inhibitors which will block the action of MMPs in arthritis. Predictive, high through-put enzymic assays are essential for inhibitor identification. Ideally, such assays must be convenient, kinetically predictive, and flexible to accommodate the numerous candidate MMPs potentially involved in cartilage degradation. Thus, we have evaluated several MMP assays for inhibitor assessment and these are detailed below.

Our initial assay was based upon proteolysis of ^{3}H-transferrin (H Nagase, personal communication). This assay employed detection of protease cleavage products based upon the extent of solubilization of TCA precipitable radioactivity initially present in the transferrin substrate. The advantages of this assay were its broad utility toward several proteases and ease of detection of enzymic activity present in cartilage extracts or culture medium. Although this assay gave linear kinetics with respect to time and enzyme, its linearity with respect to substrate was anomolus.

The initial proteolytic cleavage of transferrin causes formation of additional peptides which can also serve as substrates for the desired protease. Thus, its utility for determining kinetic parameters could not be rationalized. Also, the need for an assay which employed a substrate with a single cleavage site giving rise to a single product which would not interfere with subsequent protease activity on the initial substrate became evident.

A second approach to the desired assay employed a peptide substrate with HPLC detection of reaction products (Harrison et al. 1989). In this assay Substance P, an 11 amino acid peptide, was used to assess stromelysin (MMP-3) activity. MMP-3 cleaves Substance P at the Gln-Phe peptide bond between residues 6 and 7. The advantage of this assay was the high degree of specificity seen with MMP-3 and its amenability to providing solid kinetic data upon which inhibitor design could be based. Unfortunately, the high degree of specificity was also a shortcoming of this assay as it could only be used for MMP-3. In addition, the cumbersome HPLC separation protocol did not allow this assay to be used in a high capacity screening format.

Evolution of our MMP assay was greatly aided by the characterization of a synthetic, fluorogenic peptide substrate which enabled a continuous assay protocol to be developed (Knight et al. 1992). This peptide which we refer to as the MCA peptide (7-methoxy-coumarin-4-yl)Acetyl-Pro-Leu-Gly-Leu-(3-[2,4-dinitrophenyl]-L-2,3-diaminopropionyl)-Ala-Arg-NH_2 takes advantage of the highly fluorescent 7-methyoxycoumarin group which is quenched by energy transfer to the 2,4-dinitrophenyl group in the intact peptide. However, upon cleavage by numerous MMPs (MMP-1, 3, 8, 9, PUMP), an approximate 200-fold increase in fluorescence is observed due to liberation of the MCA-containing peptide fragment (λex 328 nm, λem 393 nm). The broad susceptibility of this peptide to cleavage by numerous MMPs, along with its amenability to a high capacity, continuous assay format makes it ideal for evaluating MMPs which may be involved in cartilage degradation. Michaelis-Menten kinetic constants have been determined for MMP-1, 3, 8, and 9. The Vmax values are 0.93, 1.10, 9.70 and 0.22 fluorescence units/min/ng protein. The Km values are 9.8, 8.2, 4.4, and 2.7 mM, respectively. We are currently using this assay to evaluate various inhibitors which have been described to inhibit the MMP family of proteases.

References

Harrison R K, Teahan J, Stein R L. A semi-continuous HPLC-based assay for stromelysin. Anal. Biochem 1989; 180: 110–3.

Knight C G, Willenbrock F, Murphy G. A novel coumarin-labelled peptide for sensitive continuous assays of the matrix mettaloproteinases. FEBS 1992; 296: 262–6.

Development of a convenient and sensitive assay for matrix metalloproteinase enzyme activity in synovial fluid samples using fluorigenic peptides

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Matrix metalloproteinases (MMPs) are considered to play a major role in the proteolytic degradation of extracellular matrix in joint and skeletal diseases. Inactive forms of these enzymes are secreted by cells of the joint, together with their tissue inhibitors (TIMPs). Extracellular activation results in active MMPs which can degrade most matrix components of articular cartilage such as proteoglycans and collagen proteins. Matrix degradation is inhibited by interaction of TIMPs with active MMPs. Under physiological conditions, active MMPs are efficiently neutralized, predominantly by TIMPs (MMP/TIMP ratio ≤ 1), so that net proteolytic activity is absent or very low. Under pathological conditions like in rheumatoid arthritis and osteoarthritis, levels of active MMPs as well as TIMPs are increased. More importantly, active enzymes are present in excess over the amount of tissue inhibitor (MMP/TIMP ratio>1).

The actual deleterious species in proteolysis are active MMPs, free of inhibitor. Therefore, ideally assays to monitor the potential for tissue degradation should represent net enzyme activities of MMPs rath-