Biomarkers of joint disease, injury and repair: Where are we?

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There is a pressing need to develop and validate reliable biochemical markers that can inform on the processes of joint destruction and remodeling in osteoarthritis (OA) and other conditions (1). Such markers would aid in drug development for OA by identifying patients who are fast progressors and detecting an early response to therapy and so reducing patient numbers and time required for clinical trials. The NIH-sponsored Osteoarthritis Initiative (web link: http://www.oai.ucsf.edu/) is a nationwide research study and clinical sample resource that has recognized the need to promote biomarker development. Joint cartilage biomarkers would also be useful for monitoring the consequences of joint injury and repair processes after surgical intervention. Collagen degradation products have proven valuable clinically as biomarkers of bone turnover and continue to have promise in the study of joint disease (2). Here we summarize some of the progress and the challenges.

Collagen Type II as a Cartilage Degradation Biomarker

Body fluid markers of cartilage metabolism have received much research attention, in particular type II collagen breakdown products (3). Since type II collagen is largely restricted to hyaline cartilages, it presents an attractive target as a potential monitor of joint erosion processes. Besides face validity, however, it is essential to establish any candidate marker by unequivocal molecular identification in biological samples as a prelude to wide-ranging, potentially misleading clinical studies of markers that are still experimental. It is notable that of the several collagen type II peptide-based immunoassays in clinical research, none have as yet been validated by definitive direct methods to establish the molecular nature of the signal generators in adult serum and/or urine (4).

The problem so far has been a lack of application of direct methods for validating candidate molecular targets that are largely being designed theoretically. Antibodies have tended to be raised and developed into assays using synthetic standards, validated at best with tissue-derived samples, then deployed in wide-ranging cross-sectional clinical studies without adequate validation that the signal source in vivo is correct, not a cross-reactivity. Differences between patient and control groups are taken at face value, interpreted in terms of the intended target and the published reports used to recruit further clinical research collaborations without pursuing the more challenging but essential molecular validation. The scientific bar needs to be raised with a focused strategy of screening, identification and molecular validation of marker epitopes beyond simply face validity of the intended target degradation products.

The impact, if a product of joint cartilage destruction can be identified that is disease-process specific and hence has a high signal to noise and OA patient to non-OA patient ratio, will be several-fold. First, and perhaps foremost, the chief benefit will be in drug trials (at every level: preclinical phase I, II and III). A longer term and more profound
impact will be in aiding in the clinical stratification and management of individual
patients. Of particular importance is the potential to develop a biochemical marker(s)
that can detect changes in joint cartilage(s) early in the process of a disorder that can
lead to joint failure before even the most sensitive imaging methods (e.g., research MRI
protocols) can reveal abnormalities, for example soon after joint injury.

One strategy we are pursuing is to apply advanced mass spectroscopic methods to
identify all collagen fragments in the human urinary peptide pool (urinary proteome) to
reveal the best molecular candidates for a new generation of markers (5). We believe
this will lift the skeletal tissue biomarker field to a higher scientific standard by providing
reference data through direct, definitive analyses. In research terms the value will be in
saving wasted effort in pursuing unvalidated, non-informative markers and focusing
assay development efforts on molecular targets identified directly by unequivocal
methods. The health care benefits are both short-term in providing the drug and
biotechnology industries with science-based qualified biomarkers to aid their efforts in
drug development and long-term with the promise of new screening tests to detect early
disease onset, assess the burden of disease activity, predict risk of progression and
monitor response to therapy in the individual patient.

**Collagen type III as a repair molecule in articular cartilages**
Collagen type III accumulates in mature articular cartilage, and can become particularly
prominent in adult human joints (6). We isolated the pool of type III collagen from
human articular cartilage samples by protease digestion. Using mass spectrometry,
purified peptides were shown to have originated from a cross-linking interaction
between collagen type III and collagen type II molecules (7). Published reports have
shown by transmission electron microscopy that type III collagen is located on the
surface of type II collagen fibrils and concentrated pericellularly (8). We conclude that
the chondrocytes can express type III collagen, which polymerizes and cross-links to
the surface of the existing collagen type II network. Using antibodies to the N-propeptide
domain we could also show that most of the type III collagen is in the form of pN-type II
molecules. These findings support a concept that collagen type III is made in cartilage
as in many tissues to provide a temporary supporting scaffold for the matrix during new
tissue growth and/or remodeling after injury. It may serve a related function in the
collagen type II-based fabric of adult cartilages to that in collagen-type-I-based soft
connective tissues in general.

By studying the pattern of collagen cross-linked peptides, the intimate, covalent
relationship between collagens II and III was revealed (7). Telopeptides from both N-
and C-terminal ends of type III collagen were linked in the tissue to helical cross-linking
sites in type II collagen. Reciprocally, telopeptides from type II collagen were recovered
cross-linked to helical sites in type III collagen. Cross-linked peptides were also
identified in which a trifunctional pyridinoline linked an $\alpha$1(II) telopeptide to an $\alpha$1(III)
telopeptide to an $\alpha$1(III) helix. This could only have arisen from a cross-link between
three different collagen molecules, types I and III in register staggered by 4D from
another type III molecules. Type III collagen is known to be prominent at sites of healing
and repair in skin and other tissues. The new findings extend the role of type III
collagen, which is known to be synthesized and deposited in mature articular cartilage (9,10), as a covalent modifier that may add cohesion to the existing fibril network presumably as part of a healing response to tissue injuries and matrix damage (6,10). Indeed, in ongoing studies, we have found significantly higher levels of collagen type III in normal appearing full-thickness cartilage from osteoarthritic hips compared with age-matched control tissue from hip fracture cases (11).

**Urinary collagen peptides**

Using mass spectrometry/liquid chromatographic methods, we can profile cross-linked collagen fragments from tissue breakdown in urine. We originally discovered discrete pools of collagen telopeptide domains in urine (12) that have become the bone resorption markers of choice in clinical trials of osteoporosis drugs and in patient management (2). A type II collagen fragment in urine we identified as a potential biomarker (13) has been targeted in a commercial assay(1,3) that is reported to reflect joint cartilage destruction in clinical studies. Direct scientific validation that the immunoassay is indeed responding to the intended target epitope in urine or serum is still lacking.

Cross-linked collagen peptides survive into urine because the cross-link protects adjacent short sequences from proteolysis resulting in discrete peptides rather than a broad spectrum (12). This explains their strengths as tissue degradation biomarkers. Using new and definitive mass spectrometric methods, we are targeting biomarkers of bone collagen post-translational quality (as a potential risk factor for bone fragility) and cartilage collagen fragments with the goal of identifying more specific markers of joint cartilage breakdown than the current generation of research assays in the literature.

In summary, developing clinically useful molecular biomarkers of joint cartilage remodeling and osteoarthritis (OA), a disease for which there are no effective drugs, has proved to be a major challenge (14). Efforts in the last 10-20 years have produced a range of candidate biomarkers but none have proven to be convincingly useful clinically. Though the literature is becoming saturated with publications from cross-sectional biomarker studies, the “noise” is becoming counterproductive and, indeed, responsible for a growing belief that useful OA biomarkers are unlikely (14). We are more positive and believe that a focused application using targeted proteomic mass spectrometry can move this field forward.

**References:**


