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Functional genomics in identification of drug targets in Dupuytren's contracture

Mirela Sedic[1], Davor Jurisic[2],Zdenko Stanec[3], Karlo Hock[1], Kresimir Pavelic[1,4], Sandra Kraljevic Pavelic[1,4]

[1]Rudjer Boskovic Institute, Division of Molecular Medicine, Laboratory for systems biology, Bijenicka c. 54, 10000 Zagreb, Croatia, [2]University Hospital Center Rijeka, Clinic for Surgery, Department for Plastic and Reconstructive Surgery, Kresimirova 42a, 51000 Rijeka, Croatia, [3]University Hospital Dubrava, Clinic for Plastic, Reconstructive and Aesthetic Surgery, Avenija Gojka Suska 6, 10 000 Zagreb, [4]University of Rijeka, Department of Biotechnology, Trsat, 51000 Rijeka

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1. ABSTRACT

Although functional genomics methods offer new viewpoint on molecular processes involved in particular pathological state, these methods, in particular proteomics, are still under-represented in Dupuytren's contracture research. However, several recent papers based on functional genomics technologies represent a breakthrough in studying Dupuytren's contracture as they revealed new molecular players that had been impossible to detect by traditional molecular biology methods. Using computational tools to provide biological context for such broad arrays of data accelerates the process of homing in on the potential molecular markers and drug targets. Interactomes, maps of protein-protein interactions characteristic for the disease and as such putative models of its molecular pathology, are especially useful for this purpose, facilitating the transition from global screening methods to specific experiments aimed at therapy development. The combination of these approaches in Dupuytren's contracture research might therefore facilitate the discovery of novel therapeutic targets and diagnostic markers indicative of disease progression.

2. INTRODUCTION

Dupuytren's contracture (DC) is a palmar fibromatosis that affects one or more digits, ending as an irreversible, permanent and progressive contracture with concomitant loss of hand function. One of the major histological features of Dupuytren's contracture, differentiation of fibroblasts into myofibroblasts, is considered to be responsible for the development of typical disease symptoms, and this process is driven by TGF-β (tumor growth factor-β). It is generally accepted that myofibroblasts produce contractile force *via* continuous association of the cords within cellular microfilaments of myofibroblasts. Such distinct junction site is called fibronexus and is located at the surface of myofibroblasts in the afflicted tissues (1). The fibronexus is composed of actin, myosin and associated protein filaments (*e.g.* vimentin and desmin), and extracellular fibronectin filaments at the plasma membrane. Once myosin is activated, contractile force is transduced from intracellular actin microfilaments to extracellular collagen cords, leading to progressive contracture of the affected fasciae (2).

Table 1. Molecular factors known to be involved in development of Dupuytren's contracture

Growth factors	TGF-α and TGF-β EGF (Epidermal growth factor) PDGF (Platelet-derived growth factor)
Growth factor receptors	EGF-R (Epidermal growth factor receptor)
Transcription factors	Zinc finger protein 9
Metalloproteinase enzymes and their inhibitors	MMP-2 (Matrix metalloproteinase 2) TIMP (Tissue inhibitors of metalloproteinases)
Extracellular matrix components	Collagen type III (immature form) Proteoglycans Fibronectin
Immune system	Interleukins Interferons GM-CSF (Granulocyte-macrophage colony-stimulating factor)
Oxidative stress	Oxygen free radicals

Clinically, Dupuytren's contracture is manifested through two structurally distinct forms, the nodule and the cord (3). It is, however, still not clear whether the fibrotic process induces coordinated formation of both nodules and cords (4, 5), or whether nodules and cords represent different stages of the disease and are formed independently of each other (6). Even so, modern classification of the disease progression proposes three developmental phases (7). The main feature of first or proliferative phase is the proliferation of nodular fibroblasts and their subsequent differentiation into myofibroblasts (8). Myofibroblasts combine morphological features of fibroblasts and smooth muscle cells, and are characterized by the expression of α-smooth muscle actin (9). Proliferative phase is succeeded by involutional phase in which myofibroblasts are arranged along the tension line in cords. Terminal phase of the disease, termed residual phase, is recognized as the stage in which the total number of cells in the tissue decreases, and is therefore also known as the acellular phase characterized by specific histological, 'scar-like' appearance of the tissue with thick cords of collagen and fibrocystic cells.

Many environmental factors have been associated with development of Dupuytren's contracture, including smoking (10), alcoholism (11), epilepsy or anti-epileptic drugs (12), diabetes mellitus (13), arcus corneae, hypercholesterolemia, rheumatoid arthritis (14), Carpal Tunnel Syndrome (CTS) (15), manual labor, trauma (16) and HIV infection (10). Although there is a high degree of correlation between the aforementioned factors and the occurrence of the disease, their connection with the development of clinical symptoms is still vague. Further on, a pronounced familial component has been recognized in the etiology of Dupuytren's contracture. Hereditary predisposition can thus play an important role in the pathogenesis (10).

3. MOLECULAR FACTORS INVOLVED IN DEVELOPMENT OF DUPUYTREN'S CONTRACTURE

One of the possible genetic factors that might be responsible for development of the disease is TGF-β (Table 1). Aberrant growth factors expression, *e.g.* of TGF-β and TGF-α, known to be an extremely important event in all types of fibrosis, is believed to drive at least two molecular processes in Dupuytren's contracture: A) proliferation of fibroblasts and their differentiation into myofibroblasts (17), and B) production of dense extracellular matrix containing elevated levels of fibronectin, type III collagen and proteoglycans (2). However, there is no current evidence proving the association between the common TGF-β 1 and TGF-β 2 polymorphisms and Dupuytren's contracture (18). Several authors have reported that the expression of unstable form of zinc-finger protein 9 (Zf9) could predispose patients for development of Dupuytren's contracture (Table 1), as this protein seems to be directly responsible for increased synthesis of TGF-β 1 and 2 and their receptors in serum and tissue (18). The gene coding for this transcription factor therefore represents a potential candidate for investigating genetic susceptibility to this disease. Indeed, Bayat *et al.* genotyped a novel single nucleotide polymorphism in the 3' untranslated region of the Zf9 gene in the study following up a cohort of 138 patients with Dupuytren's contracture. The results indicated that the presence of the G allele versus the A allele is associated with an increased risk of developing Dupuytren's contracture (19).

Aside from growth factors and their respective receptors, several molecular factors, including, transcriptional factors, metalloproteinase enzymes and their inhibitors, extracellular matrix components, immune system components and oxidative stress response elements are involved in the DC development (Table 1). Dense extracellular matrix over-produced by myofibroblasts and containing fibronectin, laminin, collagen and tenascin as major constituents, represents one of the major biochemical features of the palmar fascia affected by Dupuytren's contracture (20). Normal palmar fascia contains mainly type-I collagen. On contrary, type-III collagen, which is virtually absent from normal adult palmar fascia, predominates in the tissue of patients with Dupuytren's contracture (21). After quick synthesis of immature, normal-length type-III collagen, contractile force of myofibroblasts causes collagen structure of tissues to shorten, leading to an increase of contractile force and loss of hand function. This process is believed to be induced by increased density of fibroblasts, stimulation of fibroblast growth by growth factors, decreased rate of apoptosis (programmed cell death) and disproportion between the amounts of collagenase (also known as matrix mettaloproteinase) and matrix mettaloproteinase inhibitors (tissue inhibitors of mettaloproteinase, TIMP) (2). Besides type-III collagen, Dupuytren's contracture tissues also contain abundant amounts of fibronectins, glycoproteins ubiquitously found in connective tissue produced by fibroblasts, platelets and keratinocytes.

Several authors have drawn parallels between Dupuytren's contracture and wound healing. Howard *et al.* have propounded that Dupuytren's contracture might be an exaggerated form of wound healing (22). This hypothesis is based on the elevated levels of two important wound-healing-associated proteins, Hsp47 (heat shock protein 47)

and adult and oncofetal fibronectin (IIICS spliced variant), present both in clinical tissue samples and primary cell cultures from Dupuytren's contracture patients. Furthermore, Neumüller *et al.* established a significant association between palmar fibromatosis and increased levels of HLA-DR3 (human leukocyte antigen-DR3 complex) (23) (Table 1), and proposed that, in analogy to other diseases with autoimmunologic features, the presence of HLA-DR3 could indicate a higher risk for the formation of connective tissue autoantibodies (23). The generation of the latter is stimulated by the components of the extracellular matrix, whereby autoantibodies induce the release of profibrotic cytokines from the immune system cells.

Cytokines are small proteins that transmit signals between the cells and participate in the processes activated during injuries and infections. Their production rises under different pathological conditions, including the cellular response to oxygen free radicals (2). The most preponderant cytokine in Dupuytren's fasciae is interleukine-1 (IL-1) (Table 1) (24). It stimulates platelets and macrophages giving rise to the secretion of growth factors, in particular TGF-β, fibroblast growth factor FGF and platelet-derived growth factor PDGF (Table 1). This process occurs along with direct stimulation of fibroblast proliferation and activation of Langerhans cells in the epidermis. Therefore, it seems reasonable to believe that immunologic response plays an important role in the pathogenesis of Dupuytren's contracture. Moreover, recent studies elucidated the mechanism by which IL-1 stimulates epidermal Langerhans cells in Dupuytren's contracture. Once activated, these cells migrate to dermo-epidermal junction and to the nodules in affected tissues (25). Langerhans cells induce cellular immunologic response, which accounts for the presence of inflammatory cells in the fascia affected by Dupuytren's contracture. In turn, inflammatory cells produce different growth factors, including TGF-β (26), leading ultimately to palm contracture (27). Increased secretion of IL-1, TGF-β and fibronectin in Dupuytren's contracture elevates the number of inflammatory cells that can also themselves stimulate production of cytokines and growth factors. In order to allow these events to take place, apoptosis in the tissues affected by Dupuytren's contracture should be either completely abrogated or partially deregulated. This seems plausible, as Jemec *et al.* found that histological specimens of the affected fasciae were not characterized by apoptotic cells (28). Furthermore, Meek *et al.* suggested that sustained myofibroblast proliferation might be the consequence of reduced rate of apoptosis of inflammatory cells present in Dupuytren's tissue (29).

Another molecular mechanism that might participate in the pathogenesis of Dupuytren's contracture is oxidative stress in combination with localized ischemia of the palmar fascia. Murrell *et al.*, who found a six-fold increase in hypoxanthine concentrations and detected xanthine oxidase activity in affected palmar fasciae (30), first brought up this possibility. Xanthine oxidase catalyses the conversion of both hypoxantine to xanthine and xanthine to uric acid, with the concomitant release of superoxide free radicals (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-). At lower concentrations, *i.e.* concentrations similar to those likely to occur in Dupuytren's contracture, oxygen free radicals were shown to have a stimulatory effect on the proliferation of cultured fibroblasts derived from the palmar fasciae of patients both with and without Dupuytren's contracture (30). Stimulated proliferation of fibroblasts might then partially account for the increase in type-III collagen typical for this disease. Another potential source for high levels of reactive oxygen species in Dupuytren's contracture tissues might be defective mitochondria, in which free radicals are generated by means of electron leak. Bayat *et al.* reported for the first time on the mutation within the mitochondrial genome (16s rRNA region) in patients with Dupuytren's contracture (31), which underlines the importance of impaired mitochondrial function in the disease pathogenesis.

4. TAKING A STEP FORWARD IN ELUCIDATING MOLECULAR PATHOGENESIS OF DUPUYTREN'S CONTRACTURE: FUNCTIONAL GENOMICS

Evidently, the molecular background of Dupuytren's contracture involves many different biochemical and cellular processes that form complex interactions in a cascade-like fashion to promote disease progression. So far, the studies have focused mainly on how a single molecular factor or process contributes to the overall pathogenesis. However, although such approach yielded valuable information on disease progression at different molecular levels, it has neither managed to provide a better context-dependent understanding of complex signaling cascades inside and outside of the affected cells, nor did it reveal the temporal sequence of these events. A global approach offered by functional genomics may however provide the answers to these unresolved issues. Functional genomics puts forth a systematic effort to identify biological pathways and processes in both normal and diseased states by using high-throughput and large-scale methodologies combined with statistical and computational analyses of obtained results (32). As such, functional genomics encompasses two different methodological approaches, namely transcriptomics and proteomics. Transcriptomics, the study of the complete set of transcripts (mRNA molecules) expressed by the genome at any given time, represents a global way of looking at gene expression patterns. Similarly, proteomics is the study of an overall complement of proteins expressed by the genome, and it covers all aspects of protein properties on a large scale including its level of expression, protein folding and 3-D structure, function, protein interactions, cellular localization and modifications (33). For a detailed overview of transcriptomic and proteomic methods, readers are referred to dedicated literature on this subject (34, 35).

There are a number of different methods for transcriptomic measurements, but currently the most popular one is the use of DNA microarrays or 'gene chips', which allow for a simultaneous detection and quantification of thousands of transcripts. Isolated RNA from cells or tissues and reference RNA are reversely transcribed into

cDNA and labeled with two distinct fluorescent dyes (*e.g.*, Cy3 and Cy5). The labeled cDNAs are pooled together to provide the so-called 'target' mixture. The target is then hybridized to the 'probe' (complementary DNA sequences immobilized on a solid surface in an ordered array) and visualized with laser-based detector measuring the fluorescence of the two dyes at each probe position. An intensity ratio between the two fluorescent dyes is calculated for each probe position, indicating the relative amount of RNA for the particular probe in both investigated and reference samples. Nowadays, the most commonly used type of DNA microarrays is the oligonucleotide array for which short 25-mers are synthesized *in situ* by photolithography onto silicon wafers (GeneChip technology, Affymetrix). DNA microarrays have proven to be very useful in an early identification of a disease, when one can observe gene expression levels characteristic for a particular disease while clinical disease symptoms are still absent. In addition, by identifying gene expression levels associated with either better or worse outcomes, or with higher or lower values of particular disease phenotype, it is possible to classify subgroups of a disease.

Transcriptomic approach has already been adopted in the research on Dupuytren's contracture, as substantiated by several papers dealing with the profiling of the tissues derived from Dupuytren's contracture patients using DNA microarrays (36-38). Qian *et al.* set out to profile Dupuytren's contracture nodules against the unaffected adjacent tendons, and found 16 and 3 genes to be up- and down-regulated, respectively, in the nodules (37). Some molecular events that can be linked with the identified over-expressed genes in the nodules include collagen degradation, generation of the contractile force, myofibroblasts differentiation, oxidative stress, regulation of apoptosis, proteolysis and inflammation, fibrosis and ossification. Although Qian *et al.* study uncovered a broad spectrum of diverse cellular processes that might account for the development of typical disease symptoms, many of these processes are not novel to the disease pathogenesis. A more comprehensive transcriptomic study was recently done by Rehman *et al.* (38). This group of authors conducted a well-designed study based on comparing the gene expression profiles between Dupuytren's contracture and transverse carpal fascia of control subjects (external control), as well as Dupuytren's contracture cords and nodules from the palm *vs.* the unaffected, patient-matched transverse palmar fascia (internal control). Besides confirming the genes previously reported in other similar studies (*e.g.* genes participating in ossification, genes coding for key collagenases and those bearing collagenolytic-like activity) (36, 37), Rehman *et al.* identified the genes in the context of respective biological pathways, resulting in the discovery of several pathways novel to the Dupuytren's contracture. Such approach revealed changes in molecular processes implicated in proteolysis, cytoskeletal development, lipid metabolism and inflammation. Detailed biological analysis of the transcripts whose expression was altered in this study pointed towards deregulation of developmental processes including cell growth, proliferation, differentiation, regulation of cell death, biological cell adhesion, localization, extracellular matrix-receptor interaction and cell communication (38). Particularly interesting finding was that Dupuytren's contracture shares some common gene expression patterns with liver fibrosis, namely over-expression of collagen type V α2 (COL5A2), ADAM metallopeptidase domain 12 (ADAM12) and cysteine and glycine-rich protein 2 (CSRP2), along with down-regulation of procollagen C-endopeptidase enhancer 2 (PCOLCE2) and matrix metallopeptidase 3 (MMP3). In addition, the authors noted a marked difference in the expression status between the major deregulated genes from the nodules and from the cords in comparison with external control fascia, which underscores the potential of transcriptomic profiling for deciphering Dupuytren's phenotype.

Although transcriptomic studies can reveal the expression status of genes and can thus partially elucidate genetic background of particular pathological condition, they cannot provide information on biological activity of proteins, as expression, structure and function of the latter could be controlled at many different regulatory points (transcription, mRNA splicing, translation, protein modifications, formation of protein complexes etc.). In addition, proteins are the major players of the signaling pathways that often overlap or interact to form complex biological networks, and neither these interactions nor the regulation patterns of related pathways can be inferred from an exclusive analysis of the transcriptome. In light of this fact, proteomics has emerged as a powerful tool complementing transcriptomic studies that can successfully help in elucidating pathobiochemical bases of processes involved in the particular disease. Proteomics covers a broad range of different methodological paradigms (39), but two-dimensional gel electrophoresis (2-DE) coupled with mass spectrometry (MS) is the most frequently employed one. In 2-DE, the mixture of proteins is first separated by isoelectric focusing and then by sodium dodecyl sulphate – polyacrylamide gel electrophoresis (SDS-PAGE). After gel image analysis, differentially expressed protein spots are excised from the gel and digested with trypsin to produce a set of peptide fragments specific for each individual protein (peptide 'fingerprint'). These peptides are then analyzed by a mass spectrometer that measures their respective masses. The protein identification follows, comparing experimental peptide masses with theoretical masses of tryptic peptides generated *in silico* from all the proteins in a protein sequence database (*e.g.* SWISSPROT, NCBI, HGMP etc.). Several different types of mass spectrometers can be used for proteomic measurements. However, MALDI-TOF (matrix-assisted laser desorption/ionization – time-of-flight) mass spectrometer is commonly employed for protein identification by peptide mass fingerprinting. With MALDI-TOF, the sample (peptide mixture) is mixed with an UV-absorbing matrix compound (*e.g.* α-cyano-4-hydroxy-*trans*-cinnamic acid) and crystallized. The mixture is then excited with a laser, causing evaporation of the matrix compound that carries the sample molecules into the gas phase. The resulting peptide ions (predominantly singly charged) are directed into TOF mass analyzer, where

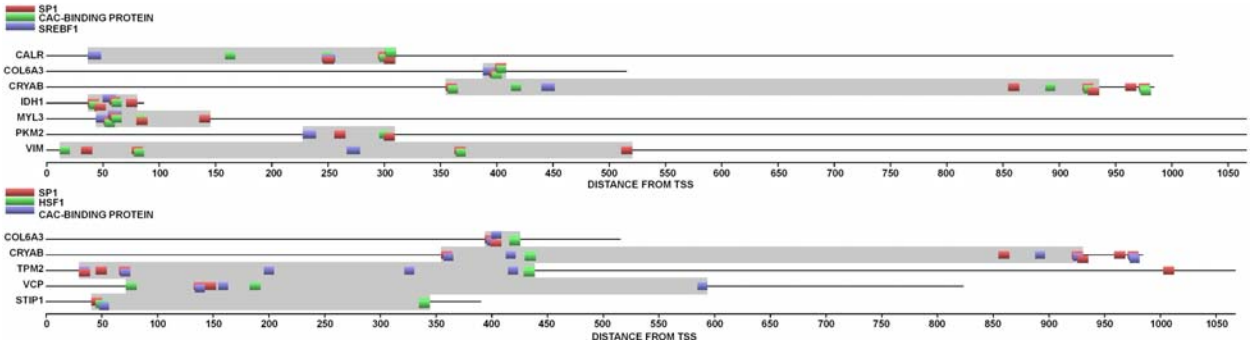

Figure 1. Hypothetical prediction of common regulatory mechanisms that control expression of the genes involved in Dupuytren's contracture pathogenesis. Differentially expressed proteins between affected and unaffected patient-matched tissue samples (41) were used to create the sets of genes that were entered into the CREME database. Two possible clusters of genes that are co-regulated at the transcriptional level were identified: one including the transcription factors SP1, CAC-binding protein, and the sterol regulatory element binding transcription factor 1 (SREBF1), and the other including SP1, CAC-binding protein, and heat shock transcription factor 1 (HSF1). TSS – transcription start site.

peptide masses are measured by determining the time required for the ions to traverse the length of the flight tube and reach the detector.

2-DE/MALDI-TOF MS has already found its application in Dupuytren's contracture research, as illustrated by Bayat *et al. (*40), who provided a short, preliminary report on proteome profiles of the nodules, cords and transverse palmar fascia from Dupuytren's contracture patients. Our group also carried out protein expression profiling of affected *vs.* adjacent, non-affected patient-matched tissues from patients with Dupuytren's contracture in the involutional phase. Similarly to the results of Bayat *et al.*, our proteomic findings were complementary to previously mentioned gene expression profiling results (37, 38). Indeed, we detected alterations in the proteins associated with extracellular matrix (ECM) production, cell proliferation and differentiation, cytoskeleton assembly and maintenance, muscle contraction, energy production (glycolysis and citric acid cycle), regulation of apoptosis and response to oxidative stress (41). The herein identified proteins were then used to create the protein-protein interaction network (interactome), which revealed for the first time the importance of autocrine regulation through ERBB-2 and IGF-1R receptors and Akt signaling pathway in pro-survival signaling in Dupuytren's fibroblasts (41). This was additionally corroborated by Western blot and immunohistochemical analyses of the affected tissues. Thus, the integrative approach based on proteomic-derived data and associated interactomics emerged as an effective paradigm to reveal new molecular processes in the disease pathogenesis and has set new directions in Dupuytren's contracture research.

While the bioinformatics tools can aid in the interpretation of proteomics results, such analyses can also extend beyond the proteome, and identify the corresponding processes not only horizontally by condensing and organizing datasets into several interconnected pathways, but also vertically, at different levels of regulation. The proteins are vital for all cell functions, and their differential expression is therefore illustrative of the molecular pathology characteristic of the disease. However, even a complete map of protein-protein interactions may not offer an exhaustive explanation of the mechanisms that bring about the pathological changes. It is therefore intriguing to invoke the central dogma of molecular biology, and investigate where exactly the flow of information from nucleic acids to proteins veers into a disease.

To illustrate the possibilities of bioinformatics tools in this kind of analyses, we used the results of our proteomics study on Dupuytren's disease (41) to explore the possibility that common regulatory mechanisms control the genes encoding for the identified proteins at transcription level. For this purpose, we used CRÈME (http://creme.dcode.org) (42), an engine that identifies common transcription factor binding sites for a given set of proteins. In this way, we were able to identify two possible clusters of genes that are co-regulated by several transcription factors, including SP1, which is involved in the cell proliferation and differentiation processes (Figure 1). Interestingly, the silencing of this transcription factor blocks fibrogenic properties of TGF-β, as suppressing its activity broadly inhibits the ECM gene expression both *in vitro* and *in vivo (*43). However, it should be noted that such *in silico* predictions warrant definitive experimental verification, as the correlation between the abundance of mRNA and protein level may in fact be poor due to the differences in the degradation rates between individual mRNAs and proteins, as well as post-translational modifications (33). Still, targeting the extracellular matrix production, one of the major components of Dupuytern's disease pathology, *via* TGF-β signaling to impede the contracture development sounds intriguing, and deserves further exploration.

Recently, SELDI-TOF MS (Surface Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) has gained much appreciation in biomedical research for its ability to reveal potential diagnostic biomarkers in diverse complex biological specimens, such

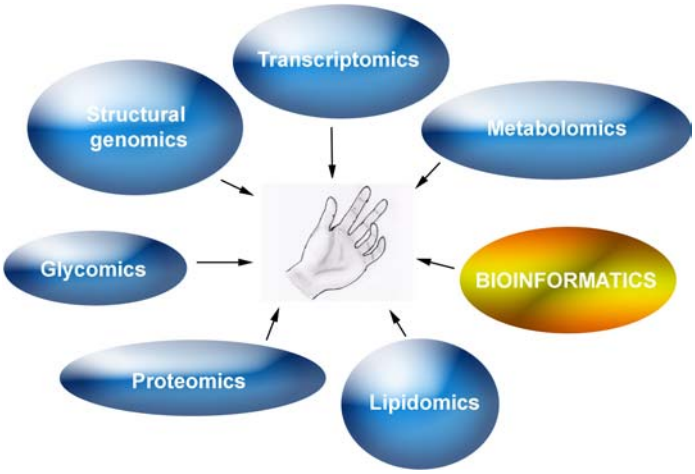

Figure 2. Functional genomics approach into studying the molecular processes underlying Dupuytren's contracture pathogenesis. The ability to combine diverse high-throughput methods from functional genomics and proteomics with bioinformatics will profoundly change the study of molecular mechanisms of diverse diseases and enable novel insights into disease pathogenesis.

as serum, plasma, intestinal fluid, urine, cell lysates, etc. In addition, this method can be used for more targeted studies (*e.g.* for characterization of protein–protein and protein–DNA interactions or post-translational modifications, namely glycosylation and phosphorylation) (44). This technique couples array-based technology (ProteinChip®, Ciphergen Biosystems Inc.) with MALDI-TOF MS. The protein chip arrays contain either chemically (anionic, cationic, hydrophobic, hydrophilic, or metal ion) or biochemically (immobilized antibody, receptor, DNA, enzyme, etc.) active surface, which retains proteins according to their specific physicochemical properties. After adding matrix solution to bound proteins, the latter are ionized with nitrogen laser and their molecular masses measured by TOF mass analyzer. As a result, unique protein abundance profiles of species bound to the chip surface are obtained. Comparisons of spectra obtained from large number of different samples reveal unique or over-expressed protein signal in a particular sample set; however, this method determines the molecular mass of differentially abundant proteins rather than their identity.

Using SELDI-TOF approach, O'Gorman *et al.* (45) detected 14 up-regulated and 3 down-regulated low molecular weight (2-20 kDa) peptides and/or proteins associated with Dupuytren's contracture. However, further stringent filtering of the protein cluster data recognized only three proteins (4600.8 Da, 10254.5 Da, and 11405.1 Da) elevated within diseased palmar fascia tissues (5.45, 11.7, and 4.28 fold, respectively) that might be directly correlated with disease progression, and thus might be taken into consideration as potential biomarkers. Although the identity of these differentially expressed proteins and peptides is yet to be established, we strongly advocate the use of SELDI-TOF-MS as a new and promising approach towards revealing so far poorly explored low molecular weight region of human proteome, as this region is potentially rich in protein markers. Implementation of novel proteomic technologies like SELDI-TOF-MS in clinical research may allow a better focus on specific molecular aberrations in diseases with obscure molecular pathogenesis, such as Dupuytren's contracture, and change the traditional ways of treatment.

5. PERSPECTIVE

Although they offer new viewpoint on molecular processes involved in particular pathological state, the functional genomics methods, in particular proteomics, are still under-represented in Dupuytren's contracture research. One of the reasons for this might be the complexity of information obtained by these methodological approaches, which might be misleading if not interpreted correctly. However, several recent papers utilizing these methodological approaches represent a breakthrough in studying this disease by revealing new molecular players that were impossible to detect by traditional molecular biology methods (Figure 2). Furthermore, recent advances in computational biology and bioinformatics can aid in the interpretation of functional genomics results. Using computational tools to provide a biological context for such broad arrays of data accelerates the process of homing in on the potential molecular markers and drug targets. Interactomes, maps of protein-protein interactions characteristic for the disease and as such putative models of processes involved in molecular pathology of a disease, are especially useful for this purpose, as they facilitate the transition from global screening methods to specific experiments aimed at therapy development. The combination of these approaches in Dupuytren's contracture research might therefore facilitate the discovery of novel therapeutic targets and diagnostic markers indicative of disease progression, and could help lay the groundwork for development of new, non-invasive treatment modalities.

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Abbreviations: DC: Dupuytren's contracture; TGF: tumor growth factor; CTS: carpal tunnel syndrome; HLA-DR3: human leukocyte antigen-DR3 complex; FGF: fibroblast growth factor; PDGF: platelet-derived growth factor; Zf9: zinc-finger protein 9; IL-1: interleukine-1; Hsp47: heat shock protein 47; 2-DE: two-dimensional gel electrophoresis; MS : mass spectrometry; SDS-PAGE: sodium dodecyl sulphate – polyacrylamide gel electrophoresis; MALDI-TOF: matrix-assisted laser desorption/ionization – time-of-flight; ECM: extracellular matrix; SELDI-TOF: surface enhanced laser desorption/ionization time-of-flight

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Send correspondence to: Sandra Kraljevic Pavelic, Rudjer Boskovic Institute, Division of Molecular Medicine, Laboratory for systems biology, Bijenicka c. 54, 10000 Zagreb, Croatia, Tel: 385-1-4571267, Fax: 385-1-4561010, E-mail: skraljevic@irb.hr

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