Understanding Dupuytren's disease using systems biology: a move away from reductionism

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INTRODUCTION

Dupuytren's disease (DD) resides within the poorly understood, yet important category of superficial quasi-neoplastic proliferative fibromatosis (McFarlane et al., 1990). This nodular palmar fibromatosis often causes permanent flexion contracture of the metacarpophalangeal (MCP) and proximal interphalangeal joints (PIPJ) of the digits (Rayan, 2007) leading to loss of function (Horner and Bralliar, 1971; Schroter, 1971; Tubiana, 1971; Rayan, 2007). DD may invade locally within the palmar aponeurosis of the hand (sparingly supplied with blood vessels). DD does not disseminate to other tissues (Seemayer et al., 1980), but, rather behaves as a benign neoplastic disorder: progressive and irreversible with a high rate of recurrence after surgical excision (current gold standard treatment for DD; Bayat and McGrouther, 2006). The increasing severity and aggressive recurrence may lead to amputation of the affected digit (Shaw et al., 2007).

Phenotypically, DD is described by its two distinct fibrotic elements: the nodule and cord. Nodules are highly vascularized, soft-tissue masses containing mostly myofibroblasts, while the cords are relatively avascular with a thickened collagen-rich structure (Rayan, 2007; Rehman et al., 2008, 2011). Different schools of thought exist regarding the definition of DD progression; they debate whether the nodule develops into cord or the two fibrotic elements occur independently from a separate precursor cell. Processes associated with DD pathogenesis include cytokines, growth factors, adhesion molecules, and extracellular matrix components (Rehman et al., 2008; Shih et al., 2009). Free radicals and localized ischemia may trigger the proliferation of DD tissue (Murrell et al., 1987). Histology has confirmed the presence of collagens, myofibroblast, and myoglobin proteins in DD, but at widely varying abundances. Pathophysiology of DD is also thought to arise either from a defect in a wound repair process or from an abnormal response to wounding (Shih and Bayat, 2010). Some of these hypotheses are non-molecular or may associate the disease with effects, rather than with a single cause. No animal model exists for the study of DD fibromatosis, yet investigations in animal studies of possibly related diseases (Akai et al., 1997) may be informative (Tart and Dahners, 1989; Kandel et al., 1991; Akai et al., 1997; Hildebrand et al., 2004). Single gene changes have correlated poorly with DD, and DD is nowadays viewed as a complex disease. We propose that DD may be a network disease, such that a systems biology approach may help its understanding.

HOW SYSTEMS BIOLOGY CAN ASSIST

Abnormal development such as in DD and cancer can influence activities within the surrounding tissues. Hence an association of these diseases with normal tissue repair processes should be expected, compromising the distinction between the genes causing the development of such a disease and the genes involved in the homeostatic response. Response to perturbations may be regulated at different levels [Daran-Lapujade et al., 2007 and processes are linked extensively, if not through metabolic or gene expression networks, then through RNAs (sense, anti-sense, or micro; Hendrickson et al., 2009)], or through dynamic ultrastructure (Westerhoff et al., 1990, 2009a; van Driel et al., 2003). To understand disease one therefore needs to look at the operation and integration of several simultaneous processes, as a function of time. Since the sum of positive (regulation) and negative (homeostasis) effects tend to decide the outcome, the approach needs to be precise experimentally and quantitative in the analysis, and relate to molecular biology and functional genomics as well as physiology (Westerhoff and Palsson, 2004).

Systems approaches have invalidated the concept of one "rate limiting" step determining metabolic pathways (Groen et al., 1982), gene expression circuits (Koster et al., 1988; Snoep et al., 2002; Stuger et al., 2002), and signal transduction (Hornberg et al., 2005a,b). A similar scenario is expected for DD, and can be tested by exploiting methodologies developed on simpler systems (Westerhoff, 2008; Westerhoff et al., 2009a,b). The impact of network theory (Barabási and Albert, 1999) on understanding has been substantial especially so in gene networks (Alon, 2007), metabolic networks (Schuster et al., 2002; Reed and Palsson, 2004; Westerhoff, 2008), in plant systems biology (Marshall et al., 2007), and even in food webs (Getz et al., 2003). Biological networks adapt and change temporally (Reijenga et al., 2005), are hierarchical in terms of space, time and organization (Westerhoff et al., 2009a), and have been optimized through evolution for multiple

properties that evade our understanding (Schuetz et al., 2007; Westerhoff, 2008; Westerhoff et al., 2009b).

New network theories are targeted more toward comprehending biological systems functionally (Molenaar et al., 2009). The Flux-Balance-Analysis objective function of maximum growth yield for instance (Edwards et al., 2002) is irrelevant for the human erythrocyte and muscle cell (Westerhoff et al., 2009b). The objective of oxygen carrying capacity plus binding capacity in the lungs and delivery capacity in the tissues, and maximum performance independent of oxygen makes more sense. It remains to be seen whether in DD the flux pattern adjusts to a new criterion of optimality, such as in some cases of tumorigenesis (Rodríguez-Enríquez et al., 2009).

If DD was triggered by a single genetic factor, then it should encounter so many diverse processes during its etiology that it will still be co-determined by the many factors regulating those processes. If the networks governing differentiation of normal fibroblasts of the palm of the hand are perturbed so as to cause differentiation into tissue that is muscle-like in terms of expressing alpha smooth-muscle actin and being contractile without the usual controllers of contraction and relaxation, then various sets of genetic perturbations could lead to DD. Here DD may be much like cancer (Hornberg et al., 2006). The dilemma is that although we now avail of an unprecedented set of methodologies for the identification and analysis of molecules in living cells, seeing more molecules may not help (Lazebnik, 2002). Visualizing the connections between them may heighten our understanding of DD.

THE WAY FORWARD: A COALITION BETWEEN SYSTEMS BIOLOGY AND MOLECULAR ANALYSES
ANATOMICAL MODEL HOOKED TO *IN SILICO* MODEL
Absence of animal models for DD can be offset to some extent by *in silico* models using integrated systems approaches. A virtual hand (Soh et al., 2009) may help elucidate functional outcomes/behaviors of the proliferation of diseased cells. Here to it should be useful to map expression patterns onto such an anatomical model and attach this to *in silico* models of metabolism and gene expression (Westerhoff, 2001). Such an

approach could overcome the ethical issues in animal research studies (Westerhoff et al., 2008).

IMAGING
Perhaps the biggest growth area in imaging technologies (Megason and Fraser, 2007) is fluorescence imaging, adapted for *in vivo* analysis. Label-free methods such as Raman microspectrometry are also showing promise (Ellis and Goodacre, 2006). The former will allow researchers to assess how components of intracellular signaling pathways interact in real time (e.g., Maeder et al., 2007; Chen et al., 2008). Computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), confocal microscopy, cell imaging, and Raman microspectroscopy already provide a platform for potentiating a knowledge base relationship between the clinician, patient, and scientist. Integration of the observation platform with dynamic-spatial models (Soh et al., 2009) has not yet been achieved. Systems biology would help evaluate the metabolic state of the various cells in the nodules and the cords of DD as a function of contractile activity and assess the de- and re-differentiation of fibroblasts and myofibroblasts.

ESTABLISH AN OPTIMUM CELL SYSTEM TO MODEL DD
Several factors need to be taken into account when building a cellular model for DD (Rehman et al., 2012). Notably, differing numbers of cell passages have been reported in the studies investigating the Dupuytren tissue, and changes in the proliferative potential of the fibroblast unstated. DD fibroblasts may possess a higher potential for matrix and collagen production through passages than control fascia cells because the DD nodules and cords result from an uncontrolled proliferative cellular state. Differences in collagen, fibronectin proteins, matrix expression proteins, and even proteoglycans could be affected by cell passage because it is thought that at earlier passages all cells would mostly be proliferating while at later passages they would tend to become senescent (Benvenuti et al., 2002) or rather quiescent.

Careful consideration should be given to factors that affect the reproducibility of data or produce instrument drift including sample preparation, instrument contamination,

and data processing. In addition to the obvious factors such as patient age, gender, disease grade, and recurrence, the time and order of sample analysis could provide significant sources of variability, potentially obscuring the biological variation which we seek to characterize.

DISCOVERY OF PATHWAY BIOMARKERS THROUGH STRESS-INDUCED STIMULI IN DD NETWORKS
Once the cell system is determined, high throughput studies can characterize disease using healthy cells to enable identification of pathways for the construction of disease-specific networks. A metabolomics approach would facilitate identification of involved metabolites, as well as the pathways in which they occur. Subsequently the integration of transcript and metabolite profiling data will determine congruence between the levels of certain metabolites, gene transcripts, and their protein product(s) to identify metabolic pathways, signaling pathways, and key networks (connections and intercellular dynamics) that may attribute to formation of DD. Next, the investigation of the cellular network response to stress-induced stimuli (e.g., response to xenobiotics) will assist the mechanistic and kinetic modeling of complete pathways. Key parameters (e.g., initial concentrations of enzymes and metabolites, enzyme kinetic properties such as K_m, k_{cat}, and K_i), and variables (e.g., concentrations of metabolites and metabolic fluxes) of the DD and control dynamical system may then be assigned.

TEXT MINING TOOLS, AND SBML MODELS FOR SYSTEMS BIOLOGY INFORMATION EXTRACTION FROM DD AND CONNECTIVE TISSUE DISORDERS
In pharma, systems biology is increasingly adopted, as is the use of next generation sequencing tools, e.g., RNA Seq, enhancing further the impact microarrays have had on transcript wide screening in the last decade to investigate disease. More than >45,000 hits in PubMed using the term "systems biology" and >54,000 hits for "DNA microarray analysis" were retrieved in early 2012. In 6 months (July, 2010 to January, 2011) 6000+ papers with the term "microarray" in title or abstract were added to the database. However only ~2100 publications on DD have appeared since the original publication by Guillaume Dupuytren in 1831.

Systems biology also tries to integrate all relevant literature data and pre-existing knowledge with new experimental data in its attempts to understand network functioning. To this aim, systematic text mining of the literature is important for extraction of experimentally validated biological entities related to DD, e.g., concentrations of metabolites, enzymes, genes, or other related disease. The rationale to develop novel DD and connective tissue-related tools, standard languages (SBML) and models may help to depict accurate relationships in bio-networks by using natural language processing approaches. Emerging SBML and various databases ranging from proteome–proteome interaction databases (Pagel et al., 2005), human metabolome database (HMDB; (Wishart et al., 2009), gene ontology (GO; (Ashburner et al., 2000) already facilitate bridging or integrating some of the existing data about DD into a knowledgebase.

THE CLINIC AND ITS LIMITATIONS

It should be recognized that increasing sample replications is not always possible, particularly for clinical samples where patients are under treatment and any new diagnostic test is a secondary goal. Therefore, there needs to be greater emphasis on more robust experimental design. For example, to avoid sample bias, adequate matching of patients with disease to those who are healthy, in terms of gender, age, BMI, and ethnicity, as well as other extrinsic factors such as metabolic rates, pharmaceutical use and diet. Multidisciplinary team are required. Transnational funding across three to four experts groups ranging from clinicians, biologists, instrumentalists, and systems biologists may allow critical mass to be established.

CONCLUSION

Approximately 181 years have elapsed since the first DD literature reference. The research has so far implicated a differentiation of fibroblasts into myofibroblasts and highlighted multiple mRNAs of which the expression level differs between diseased and healthy tissues. Extracellular matrix enzymes are involved in the disease. High throughput and candidate-gene association studies have suggested multiple biomarkers for the disease, but these would require complex and invasive sampling. Thus in

the absence of radically new findings, the present mainstream research paradigm is unlikely to lead to a full understanding of the disease. What is known about DD suggests it is a multifactorial network disease requiring targeting of a few complete sets of pathways. A part way house is needed whereby a systems biology lead is adopted and molecular data generated to test and colonize potential networks. The use of time and changes to microenvironment should be promoted. Single cell analyses too will help to assess the impact of cell location and cell–cell contact. Availability of different disease states and severities will help to define perturbations and chronograph network change and disease progression. This will also assist therapeutic target selection. A consortium effort to understand DD should be initiated and the parameters then correlated across the various levels of systemic description. This should enable a collective investigation of pathological mechanisms in DD and related fibrotic connective tissue disorders.

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