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RESEARCH ARTICLE

Interaction networks as a tool to investigate the mechanisms of aging

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Abstract Biological systems are made up of very large numbers of different components interacting at various scales. Most genes, proteins and other cell components carry out their functions within a complex network of interactions and a single component can affect a wide range of other components. Interactions involved in biological processes have been first characterized individually but this "reductionist" approach suffers from a lack of information about time, space, and context in which the interactions occur in vivo. A global, integrative, approach has been developed for several years, focusing on the building of protein–protein interaction maps or interactomes. These interaction networks are complex systems, where new properties arise. They are part of the emergent field of systems biology, which focuses on studying complex biological systems such as a cell or organism, viewed as an integrated and interacting network of genes, proteins and biochemical reactions. Aging is associated with many diseases, such as cancer, diabetes, cardiovascular and neurodegenerative disorders and this limits the investigation of the mechanisms underlying the aging process when focusing on a single gene or a single biochemical pathway. The integration of existing intracellular interaction networks with the extracellular interaction network we have developed (MatrixDB, http://matrixdb.ibcp.fr) will contribute to provide further insights into the global mechanisms of aging.

Keywords Interaction networks · Aging · Extracellular matrix

Abbreviation
GO Gene Ontology

From reductionistic to network-based approaches

Identification of genes that modulate aging, longevity or senescence is a major area of research, and a large number of genes that display age-related changes in various biological processes have already been identified (de Magalhães et al. 2009). The vast majority of biogerontological studies have focused on a single pathway and on individual genes/proteins, without

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considering the possible role of interactions between them. Indeed, biological systems are made up of very large numbers of different components interacting at various scales. Most genes, proteins and other cell components carry out their functions within a complex network of interactions and a single component can affect a wide range of other components. Interactions involved in biological processes have been first characterized individually but this reductionist approach suffers from a lack of information about time, space, and context in which the interactions occur in vivo (Barabási and Oltvai 2004). Furthermore, aging is associated with many diseases, such as cancer, diabetes, cardiovascular and neurodegenerative disorders, hence focusing on a single gene or biochemical pathway provides limited insight when investigating the mechanisms underlying the aging process. There is thus a need for an integrative, global approach to elucidate the molecular mechanisms of aging. Although there are potential biases in large-scale studies based on interaction networks, mostly due to the incomplete coverage of networks, and to the high number of interactions for the most studied proteins, network-based approaches are particularly useful methods for representing complex biological systems, and have been used by several laboratories to study aging.

If two proteins interact with one another, they usually participate in the same, or related, cellular functions and clues to the function of a protein can be obtained by seeing whether it interacts with another protein of known function (guilt-by-association, Oliver 2000). This allows to leverage existing protein annotations, typically relying on the rich structured vocabularies provided by the Gene Ontology (GO) consortium to annotate the molecular functions, biological processes and cellular locations of gene products (Ashburner et al. 2000, http://www.geneontology.org). This principle has been applied to interactomes such as the yeast interactome (Uetz et al. 2000; Ito et al. 2001), and network methods are now being applied to unravel the complexity of aging. Several potential longevity-associated genes were predicted in *C. elegans*, based on interactions with previously described longevity-associated genes (Witten and Bonchev 2007). We have recently reviewed the contribution of interaction networks to the elucidation of molecular mechanisms underlying biological processes (Chautard et al. 2009a).

Large-scale studies using gene networks and protein–protein interaction networks

The building of gene and interaction networks relies on large sets of interaction data that are stored in databases. Databases focused on aging include the Aging Genes/Interventions Database (AGEID, Kaeberlein et al. 2002) as well as the GenAge and AnAge databases (de Magalhães and Toussaint 2004) featured by the Human Aging Genomic Resources (de Magalhães et al. 2005, 2009). GenAge is a curated database of genes related to human aging (de Magalhães and Toussaint 2004), whereas AnAge is an integrative database describing the aging process in several organisms and including, if available, maximum life span, taxonomy, developmental schedules and metabolic rate (de Magalhães et al. 2005). The NetAge database (http://netage-project.org/) contains all the GenAge genes and those involved in age-related diseases that are found in the human interactome. Those data have been used to build gene networks and protein–protein interaction networks in conjunction with data stored in interaction databases such as IntAct (Kerrien et al. 2007), the Molecular INTeraction database (MINT, Chatr-Aryamontri et al. 2007), the Database of Interacting Proteins (DIP, Salwinski et al. 2004), BioGRID (Breitkreutz et al. 2008), and the Human Reference Protein Database (HPRD, Keshava Prasad et al. 2009). In interaction networks, proteins are represented as nodes that are connected to each other by edges (links), each link representing an interaction between two nodes. Several measures characterize the local or global organization of a network. The degree or connectivity of a node (protein) corresponds to the number of links for that node (number of binding partners of the protein), and the shortest path length is the path with the smallest number of links between a pair of nodes (Barabási and Oltvai 2004). Genetic determinants of longevity in *Saccharomyces cerevisiae* have been identified by applying a shortest-path network algorithm to a pre-existing protein–protein interaction dataset to construct a shortest-path network (Managbanag et al. 2008). This work is the first example of a quantitatively predictive algorithm for predicting genes/proteins that modulate longevity. The network comprised 171 genes/proteins, including 138 putative novel longevity genes, and previously unknown longevity genes have been identified (e.g. a phosphatidylinositol

4,5-bisphosphate 5-phosphatase, a mitochondrial tryptophanyl-tRNA synthetase, and a protein component of the large ribosomal subunit). This work demonstrates that shortest-path network analysis is a useful approach toward the identification of genetic determinants of longevity and represents one of the first examples where network analysis has been applied to aging and extensively validated in a biological system (Managbanag et al. 2008). The global aim of such studies is to identify protein–protein interactions that affect aging/longevity/senescence in humans, and to yield further insights into the molecular mechanisms of aging and age-associated diseases.

The first attempt towards constructing a "human longevity network" based on protein-protein interaction data was made by Budovsky et al. (2007). They selected 329 putative human longevity-associated proteins, mainly human orthologs of longevity-associated genes established in model organisms, among which 211 participate in at least one protein–protein interaction according to the BioGRID database. The resulting "core longevity network" comprises 153 longevity-associated proteins in its largest connected component. It is scale-free with an extremely high contribution of hubs (proteins with the highest number of partners) to the network connectivity. Fifteen of 17 hubs of this network are longevity-associated proteins. Non-longevity-associated proteins are very important in connecting the longevity-associated proteins within the network. 33 non-longevity-associated proteins have connections with at least five longevity-associated proteins or more. The core-longevity network was extended by including all the protein–protein interactions for these 33 nodes to give the ''extended longevity network''. Almost all of the hubs of this network are involved in at least one age-related disease (e.g. atherosclerosis, cancer, Alzheimer's disease), with many being involved in several age-related diseases (Budovsky et al. 2007). This last category includes proteins involved (i) in apoptosis (B-cell CLL/lymphoma 2), (ii) in signal transduction (catenin-beta 1, proliferating cell nuclear antigen, phospholipid scramblase 1, and spleen tyrosine kinase); (iii) in transcription (estrogen receptor 1, v-myc myelocytomatosis viral oncogene homolog, nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, signal transducer and activator of transcription 3), (iv) in cell maintenance (paxillin); and (v) in protein metabolism (ubiquitin-conjugating enzyme E2I) (Budovsky et al. 2007). Hubs are potential targets for longevity-promoting interventions.

Interactome and transcriptome data have been integrated to study the dynamic modular structure of the protein–protein interaction network during human brain aging, leading to a modular network model of aging (Xue et al. 2007). Two modules associated with the cellular proliferation to differentiation temporal switch that display opposite aging-related changes in expression have been identified. Aging might preferentially target key regulatory nodes that are important for network stability, and genes connecting different modules through protein–protein interactions (regulatory genes, tumor suppressors and oncogenes) are more likely to affect aging/longevity (Xue et al. 2007).

Interaction networks are also used to provide insights into the association between aging, longevity and age-related diseases (Wolfson et al. 2009) or cancer (Budovsky et al. 2009). Human age-related disease proteins and longevity-associated proteins together with their direct binding partners form scale-free networks, which significantly overlap one another. The common signature consists of 588 proteins, 98% of which interact between themselves defining a protein–protein interaction network common to longevity and age-related diseases (Alzheimer's disease, type 2 diabetes, and atherosclerosis). This network is enriched with signaling proteins (about 50% are signaling proteins), many of which are associated with two or more signaling pathways. Pathways associated with cell–cell and cell–extracellular matrix interactions, focal adhesion and the adherens junctions (including fibronectin, paxillin, vinculin, and Rac) are overrepresented (Wolfson et al. 2009).

The relationship between longevity and cancer has been studied to identify common genes and interactions between proteins (Budovsky et al. 2009). The protein-protein interaction network of cancer- and longevity-associated proteins forms a network of 997 proteins, and 3197 interactions. Among the 143 proteins belonging to both groups, the most connected are involved in signaling or in the regulation of transcription. Tumor suppressors are associated with pro-longevity activity, whereas oncogenes are associated with a decrease in lifespan. This study supports the existence of evolutionary parallels between longevity and cancer (Budovsky et al. 2009).

Most studies performed so far have used data from publicly available databases where extracellular matrix molecules are underrepresented. Our research interest focuses on the extracellular matrix (Ricard-Blum and Ruggiero 2005; Ricard-Blum et al. 2005) and on the extracellular interaction network and sub-networks (Chautard et al. 2009b; Faye et al. 2009a). We have developed a database (MatrixDB, http://matrixdb.ibcp.fr, Chautard et al. 2009b) collecting interactions involving at least one molecule present in the extracellular matrix. The extracellular matrix is modified during aging (Robert and Labat-Robert 2000; Robert et al. 2009), and is involved in several age-related diseases such as atherosclerosis and Alzheimer's disease where it contributes to the formation of atherosclerotic lesions and amyloid plaques, respectively.

We report here the building of an age-related interaction network focused on extracellular molecules and comprising not only protein–protein interactions, but also protein–glycosaminoglycan interactions. Most, if not all, age-related interaction networks investigated so far have been restricted to protein–protein interactions and did not take into account glycosaminoglycans that play major roles as constituents of proteoglycans, both at the cell surface where they act as co-receptors and receptors, and within the extracellular matrix (Rodgers et al. 2008).

The extracellular interaction network and aging

We have first selected extracellular proteins from the MatrixDB database (http://matrixdb.ibcp.fr, Chautard et al. 2009b), which comprises mostly protein–protein and protein–carbohydrate interactions. This database integrates data from our own literature curation, from surface plasmon resonance binding assays (Faye et al. 2009a, b), and from several interaction databases: IntAct (Kerrien et al. 2007), the Molecular INTeraction database (MINT, Chatr-Aryamontri et al. 2007), the Database of Interacting Proteins (DIP, Salwinski et al. 2004), BioGRID (Breitkreutz et al. 2008), and the Human Reference Protein Database (HPRD, Keshava Prasad et al. 2009). Gene Ontology terms and UniProtKB keywords (http://www.uniprot.org, The

Table 1 Extracellular proteins, or proteins having at least one extracellular partner, annotated with "aging", "senescence", and "age-related"

UniProt ID	Name	References (PMID number)
MatrixDB entries		
Q9Y4K0	Lysyl oxidase homolog 2	15963989
Q9UEF7	Klotho	9363890
P00441	Superoxide dismutase C [Cu–Zn]	19524016, 16782871
P04637	Cellular tumor antigen p53	11780111, 16303568, 19396980
P08294	Extracellular superoxide dismutase E [Cu–Zn]	19376805, 15974918
P27797	Calreticulin	18495355, 12763031
Q9UBX5	Fibulin-5	16120151
Q92743	Serine protease HTRA1	17053108, 17053109
P01024	Complement C3	19234341, 19168221
GenAge entries (Human Aging Genomic Resources)		
P29353	SHC-transforming protein 1	15505103, 16481327, 12571362, 10580504
P04637	Cellular tumor antigen p53	11780111, 16303568, 19396980
P00749	Urokinase-type plasminogen activator	9060969
Q9UEF7	Klotho	9363890
P01241	Somatotropin	12393957
P38398	Breast cancer type 1 susceptibility protein	11795532, 12533509
Q12805	Fibulin-3	10369267, 17872905

References linking these proteins to aging and/or senescence have been identified through literature curation

UniProt Consortium 2009) containing "aging", "senescence", and "age-related" were used to select extracellular proteins in MatrixDB. The second criterion was the existence of at least one binding partner for each selected protein. We also selected proteins from the GenAge database (de Magalhães and Toussaint 2004; de Magalhães et al. 2005, 2009), which are linked through direct evidence to aging in humans or in mammalian model organisms, and that bind to proteins or glycosaminoglycans stored in the MatrixDB database. The involvement of the selected proteins in aging was confirmed through literature curation. Interaction data stored in the MatrixDB database were used to build the interaction network of the selected proteins, which was referred to as the extracellular age-related interaction network. We queried the AGEID database (http://www.uwaging.org/) with "secreted", "extracellular", and "matrix" keywords to identify extracellular matrix genes related to aging. The two mammalian genes which met these criteria were already selected from MatrixDB and GenAge, and AGEID was not further considered in our study.

We have constructed a network with the eight and five proteins selected from MatrixDB and GenAge databases respectively, using the criteria detailed above. Extracellular superoxide dismutase E was added because the selected set of proteins contains superoxide dismutase C. The fourteen extracellular proteins, or proteins possessing at least one extracellular partner, are listed in Table 1. The interaction network of these proteins is composed of 99 proteins and four glycosaminoglycans (heparin, heparan sulfate, dermatan sulfate and chondroitin sulfate) connected by 155 interactions. It has been visualized and analyzed with Cytoscape, a free software package (Shannon et al. 2003; Cline et al. 2007), where proteins and glycosaminoglycans are represented by nodes and biomolecular interactions by links connecting two nodes (Fig. 1).

A Cytoscape plugin, NetworkAnalyzer (Assenov et al. 2008) was used to calculate the following parameters (i) the degree (or connectivity) of a node corresponding to the number of binding partners of a molecule. A molecule with more than 20 partners was referred to as a hub; (ii) the average degree; (iii) the

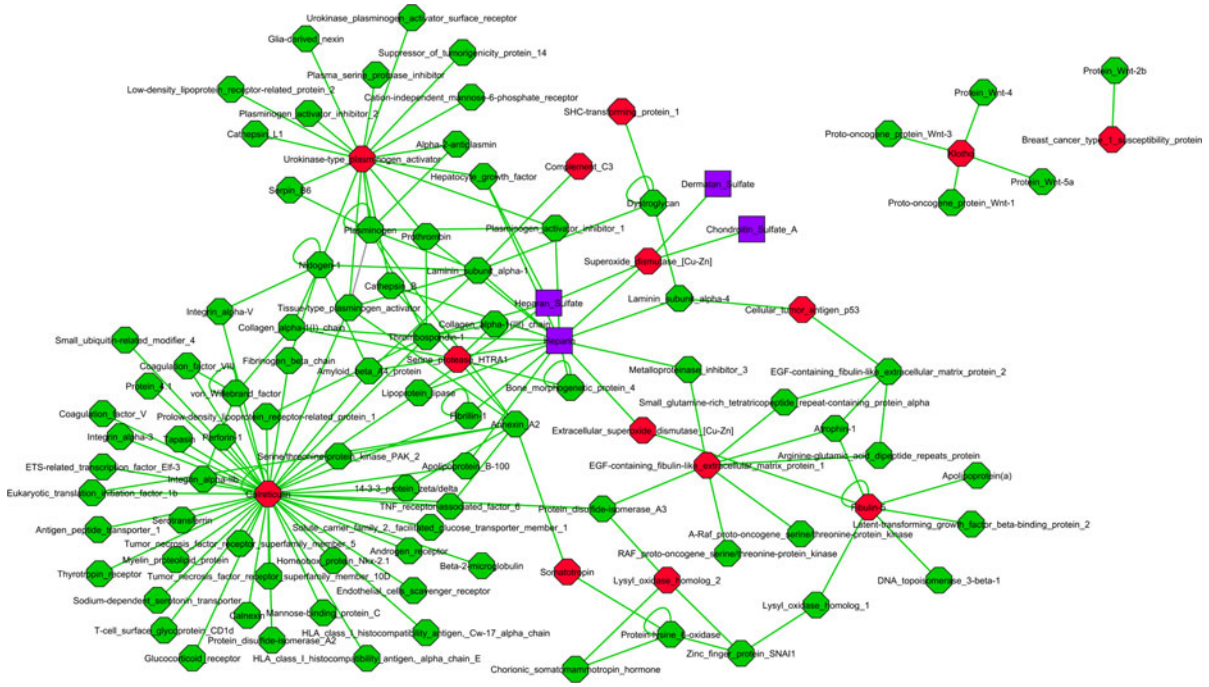

Fig. 1 Interaction network of extracellular proteins, or proteins having at least one extracellular partner, annotated with "aging", "senescence" or "age-related" (in *red*). The interaction network was visualized with Cytoscape, a free software package (Shannon et al. 2003; Cline et al. 2007). Proteins (*green octogons*) and glycosaminoglycans (*purple squares*) are represented by nodes and biomolecular interactions by links connecting two nodes. Color figure is available on-line

length of the shortest path between two nodes corresponding to the smallest number of interactions required to connect two molecules; (iv) the mean path length, representing the average over the shortest paths between all pairs of nodes (Barabási and Oltvai 2004). The most strongly connected proteins are calreticulin (44 partners, annotated with the GO term senescence) and urokinase-type plasminogen activator (17 partners, annotated in GenAge database as "affecting murine aging", with "some evidence linking it to age-related neurological diseases"). Only seven out of the 103 molecules (6.8%) are not connected to the core network (the largest connected component). The average degree is 2.83, and the mean path length is 3.39. These results show that the network is tightly connected.

The removal of molecules interacting with a single partner results in a network containing 57 molecules (two glycosaminoglycans) and 109 interactions (Fig. 2). The mean path length of this network is similar to the previous one (3.16 vs. 3.39). Only two aging-related molecules, fibulin-5 and superoxide dismutase-E, interact directly with each other. In most cases, aging-related molecules are connected through molecules which are not annotated as aging molecules (e.g. tissue plasminogen activator links calreticulin and urokinase-type plasminogen activator, and heparin links extracellular superoxide dismutase E and superoxide dismutase C) (Fig. 2). Calreticulin remains the most connected molecule of this network (degree: 18), whereas heparin is the second most-connected molecule with 15 partners.

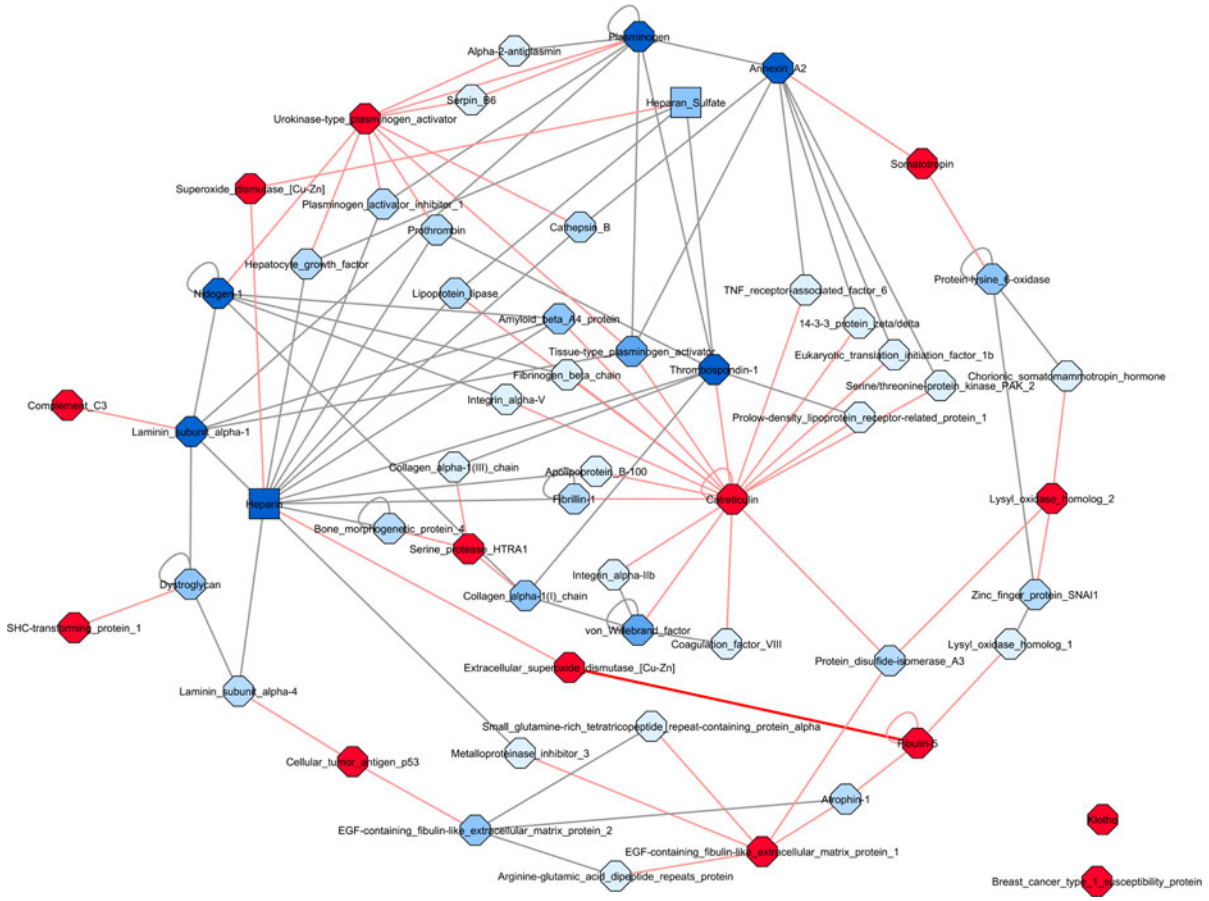

Fig. 2 Interaction network of extracellular proteins, or proteins having at least one extracellular partner, annotated with "aging", "senescence" or "age-related" and having more than one binding partner (degree > 1). Age-annotated proteins are in *red*, and their partners in *blue* (from *deep blue* to *light blue* according to their degree in decreasing order). Direct interactions between two age-annotated molecules are in *red* (*bold line*). Color figure is available on-line

This emphasizes the importance of the glycosaminoglycan in the extracellular age-related network.

Functional annotations of the extracellular age-related interaction network

The functional organization of the interaction network was characterized with Cytoscape plugins, resulting in a quantitative description of the network. Functional annotations from UniProtKB and Gene Ontology were integrated into the network as previously described (Faye et al. 2009a). A Cytoscape plugin, the Biological Networks Gene Ontology tool (BiNGO), was used to assess overrepresentation of Gene Ontology categories in biological networks (Maere et al. 2005). The Gene Ontology (GO) terms significantly overrepresented in the extended network (comprised of 99 proteins and four glycosaminoglycans) are related to protein binding, receptor binding, and calcium binding for the "molecular function" GO category (Table 2); and coagulation, response to wounding, external stimulus, and stress for the "biological process" GO category (Table 3). The above results were visualized (Supplementary material) using GOlorize, a Cytoscape plugin that exposes the Gene Ontology class structure on the network nodes (Garcia et al. 2007).

We used another Cytoscape plugin, ClueGO (Bindea et al. 2009), to decipher functionally grouped gene ontology terms. Whereas BiNGO identifies overrepresented GO terms, and reconstructs the hierarchical ontology tree, ClueGO uses kappa statistics to determine the association strength between the terms. The kappa score level threshold can initially be adjusted on a positive scale from 0 to 1 to restrict the network connectivity in a customized way. It was fixed at 0.26 for this study. Functionally grouped terms were visualized in the form of a network using ClueGO (Fig. 3). The nodes correspond to the

Table 2 GO terms ("molecular function" category) overrepresented in the extracellular aging-related interaction network, identified with the Biological Networks Gene ontology tool (BiNGO)

GO term ID	Description	Corrected *P*-value	Number of annotations in the extracellular aging network (97)
5515	Protein binding	3.5918E−12	85
5102	Receptor binding	4.0762E−11	27
5507	Copper ion binding	1.7599E−8	9
5509	Calcium ion binding	6.9222E−7	22
4866	Endopeptidase inhibitor activity	6.9222E−7	10
30414	Peptidase inhibitor activity	9.0452E−7	10
5201	Extracellular matrix structural constituent	1.0403E−6	8
4252	Serine-type endopeptidase activity	1.0403E−6	10
8236	Serine-type peptidase activity	3.6317E−6	10
17171	Serine hydrolase activity	3.6317E−6	10
8201	Heparin binding	3.6735E−6	8
4175	Endopeptidase activity	8.8243E−6	13
4857	Enzyme inhibitor activity	1.0820E−5	11
30246	Carbohydrate binding	1.6324E−5	12
4867	Serine-type endopeptidase inhibitor activity	2.2448E−5	7
5539	Glycosaminoglycan binding	2.8087E−5	8
1871	Pattern binding	4.1762E−5	8
30247	Polysaccharide binding	4.1762E−5	8
2020	Protease binding	4.5172E−5	4

97 proteins were included in the analysis because two proteins (Small ubiquitin-related modifier 4, and HLA class I histocompatibility antigen, Cw-17 alpha chain) and the four glycosaminoglycans are not annotated. The statistical significance was reached for a corrected *P*-value of 5E−5

Table 3 GO terms ("biological process" category) overrepresented in the extracellular aging-related interaction network, identified with the Biological Networks Gene ontology tool (BiNGO)

GO term ID	GO term definition	Corrected *P*-value (Maere et al. 2005)	Number of annotations in the extracellular aging network (98)
50817	Coagulation	1.9878E−11	13
7596	Blood coagulation	1.9878E−11	13
9611	Response to wounding	1.9878E−11	22
7599	Hemostasis	2.4983E−11	13
42060	Wound healing	2.3799E−10	14
50878	Regulation of body fluid levels	3.4505E−10	13
9605	Response to external stimulus	1.7590E−9	25
6950	Response to stress	1.8134E−6	30
65008	Regulation of biological quality	2.6306E−6	27
32501	Multicellular organismal process	3.7709E−6	52
7223	Wnt receptor signaling pathway, calcium modulating pathway	1.1718E−5	5
32502	Developmental process	1.5527E−5	42
42730	Fibrinolysis	1.8757E−5	4
48513	Organ development	1.8757E−5	29
50793	Regulation of developmental process	2.0292E−5	24
7275	Multicellular organismal development	3.2691E−5	39

98 proteins were included in the analysis because one of the proteins belonging to the network (P35237, Serpin B6) and the four glycosaminoglycans are not annotated. The statistical significance was reached for a corrected *P*-value of 5E−5

"biological process" category of GO terms, the relationship between the selected terms being defined based on their shared genes (Bindea et al. 2009). The biological processes represented in the aging-related extracellular network are clustered into several functional groups represented by their most significant term. This representation allows a better visualization of the groups of processes overrepresented in the network. They are related either to the extracellular matrix itself (extracellular matrix organization, regulation of multicellular organism growth, tissue morphogenesis, blood coagulation, platelet activation, and response to wounding) or to the biological interplay between the extracellular matrix and the cells (cell aging, nuclear transport, endocytosis, and cortical actin cytoskeletal organization) (Fig. 3). We have shown above that heparin is one of the two most connected molecules of the extracellular age-related network. This glycosaminoglycan, which is often used as a model for heparan sulfate in interaction experiments, regulates blood coagulation (Lever and Page 2002), which is one of functional groups identified in the extracellular age-related network (Fig. 3). The involvement of heparin/heparan sulfate in aging is further supported by the fact that heparan sulfate, the physiological counterpart of heparin at the cell surface and in tissues, contributes to biological processes such as development, extracellular matrix organization, storage of growth factors, tissue repair and wound healing, cell–cell cross-talk and adhesion, signalling, and endocytosis (Bishop et al. 2007) that are overrepresented in the functional groups of the extracellular age-related network (Fig. 3).

Conclusion and perspectives

We report here a brief overview of the advances in the field of aging-related research introduced by a global approach based on large-scale datasets and protein–protein interaction networks. The comprehensive analysis of the networks focused on aging, longevity, senescence and age-related diseases will be extremely useful to predict novel genes and pathways associated with these biological processes.

Most studies performed so far have used data from publicly available databases where extracellular matrix molecules, of major interest for aging studies,

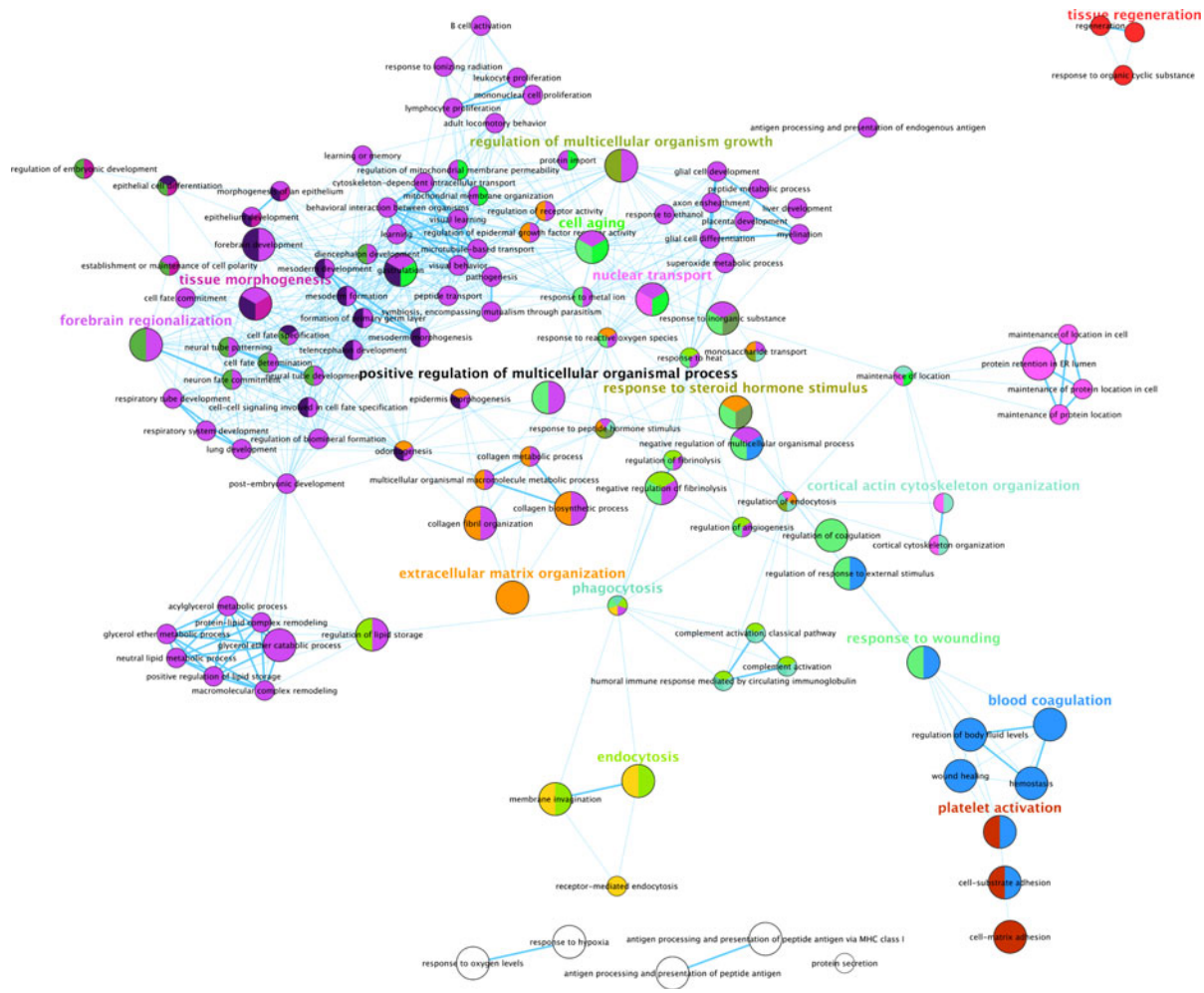

Fig. 3 Extracellular age-related network with GO terms as nodes linked based on their kappa score level (0.26), where only the label of the most significant term per functional group is shown. The node size represents the term enrichment significance (Bindea et al. 2009). Biological processes are *color-coded* as indicated on the figure. Color figure is available on-line

are underrepresented. Furthermore, these studies focused on protein–protein interactions and did not take into account glycosaminoglycans and proteoglycans that participate in signaling and in the organization of the extracellular matrix. We report here the building of an extracellular age-related network comprising both extracellular proteins and glycosaminoglycans. Among overrepresented biological processes in this network, "response to wounding" and "tissue regeneration" are related to extracellular events, which are impaired upon aging. As an example, skin atrophy is a common manifestation of aging (Callaghan and Wilhelm 2008; Uitto 2008) and is frequently associated to delayed wound healing.

Overrepresented molecular functions in the extracellular age-related network are binding to proteins, receptors, and calcium. Protein–protein interactions are indeed of major importance for the assembly and the organization of the extracellular matrix, including collagen fibrillogenesis and elastogenesis which are altered in the elderly (Uitto 2008). Cell–matrix interactions, mediated via several receptors, are modified with aging and age-related diseases, and calcium plays a crucial role in many matrix–matrix, cell–matrix and cell–cell contacts. Since a number of extracellular matrix proteins bind calcium (Maurer and Hohenester 1997), the disruption of calcium homeostasis occurring in aging and neurodegenerative

diseases might induce structural and functional changes in the extracellular matrix. In addition, calcium binds to heparin (Mattai and Kwak 1988), which is one of the two most connected molecules of the extracellular age-related network. Heparan sulfate, the physiological counterpart of heparin at the surface of the cells and in the extracellular matrix, participates in biological processes that are found in overrepresented functional groups of the extracellular age-related network. The role played by the glycosaminoglycans in aging is a key insight brought about by this study. Indeed, most studies on aging focus on genes and the changes induced in their expression level, and/or on proteins and their modifications during the aging process (e.g. glycation). These preliminary results on the role of heparin/heparan sulfate in aging warrant further investigation, but they validate the relevance of this integrative network-based approach to identify the molecular mechanisms of aging.

To introduce dynamics into the extracellular age-related interaction network, we started to determine the kinetics and affinity of each interaction using surface plasmon resonance assays. Association rates will be helpful to prioritize binding events and the value of equilibrium dissociation constants (K_D) will be used to put weight on the interaction to further hierarchize them. This integrative approach will contribute to decipher molecular mechanisms occurring within the extracellular matrix and at the cell–matrix interface, and contributing to aging and age-related diseases.

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