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Integrated procedures for accelerating, deepening, and leading genetic inquiry: A first application on human muscle secretome

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ABSTRACT

Purpose: Beyond classical procedures, bioinformatic-assisted approaches and computational biology offer unprecedented opportunities for scholars. However, these amazing possibilities still need epistemological criticism, as well as standardized procedures. Especially those topics with a huge body of data may benefit from data science (DS)-assisted methods. Therefore, the current study dealt with the combined expert-assisted and DS-assisted approaches to address the broad field of muscle secretome. We aimed to apply DS tools to fix the literature research, suggest investigation targets with a data-driven approach, predict possible scenarios, and define a workflow.

Methods: Recognized scholars with expertise on myokines were invited to provide a list of the most important myokines. GeneRecommender, GeneMANIA, HumanNet, and STRING were selected as DS tools. Networks were built on STRING and GeneMANIA. The outcomes of DS tools included the top 5 recommendations. Each expert-led discussion has been then integrated with an DS-led approach to provide further perspectives.

Results: Among the results, 11 molecules had already been described as bona-fide myokines in literature, and 11 molecules were putative myokines. Most of the myokines and the putative myokines recommended by the DS tools were described as present in the cargo of extracellular vesicles.

Conclusions: Including both supervised and unsupervised learning methods, as well as encompassing algorithms focused on both protein interaction and gene represent a comprehensive approach to tackle complex biomedical topics. DS-assisted methods for reviewing existent evidence, recommending targets of interest, and predicting original scenarios are worth exploring as in silico recommendations to be integrated with experts' ideas for optimizing molecular studies.

1. Introduction

We all are immersed into the data-explosion era, associated with the ever-expanding computing power and data access. Within this glut of information, societies and people are obviously exposed to multiple threats and opportunities. Overall, this overwhelming amount of information has been leading scholars to novel great challenges. Within this framework, biology has been overdosed by data, pushing researchers to develop novel analytics. Although the very beginnings of bioinformatics occurred many decades ago even before DNA sequencing methods, the

Abbreviations: AI, artificial intelligence; DS, data science; EV, extracellular vesicles; FN, functional gene network; PI, protein-protein interaction network; TM, text mining; XC, functional gene network extended by co-citation.

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computer-assisted analyses in biological fields, the international collaborations, and the birth of dedicated journals stabilized bioinformatics to modern science in the 1980s; then, through the genomics era, the high-throughput bioinformatics, and the collaborative computing, the bioinformatician as specialized professional has emerged massively in life sciences [1].

The possibilities offered by bioinformatics are not limited to the analyses of experimental data. Moving beyond classical procedures of the integrative review (i.e., a review approach that combines diverse methodologies, sources, analyses, and presentation of results [2]), bioinformatic-assisted approaches allow to advance the understanding of the topics under a systems biology approach [3]. Therefore, bioinformatic-assisted reviews may enable us to extract validated and meaningful information for a given biological phenomenon, as well as to analyze potential mechanisms of action [4]. It should be specified that bioinformatic-assisted review should not be confused with either technology-assisted review (otherwise called computer-assisted review) or artificial intelligence (AI)-assisted peer review, which use AI methods such as predictive coding or continuous active learning to perform faster review processes than human teams. To this end, machine learning approaches can support peer review by increasing efficiency in the quality control and peer review process [5].

Back to biology, unlocking the secrets of health and diseases, as well as unveiling the bases of biology itself and depicting novel insights through advanced computing techniques, are the core aims of computational biology. This field is currently facing the fact that biological data are diverse from other big data, since they are inherently driven by evolutionary complex processes; in addition, datasets are nowadays so massive that the extraction of patterns that give clues to biological processes is anything but simple [6]. These arguments fall into AI, that John McCarthy defined as "*the science and engineering of making intelligent machines, especially intelligent computer programs. It is related to the similar task of using computers to understand human intelligence, but AI does not have to confine itself to methods that are biologically observable*"; in this reductionist view, intelligence is considered as "*the computational part of the ability to achieve goals in the world*" [7]. AI takes advantage of data science (DS) procedures for creating systems that think and/or act like humans. AI encompasses the sub-field of machine learning, whose deep learning is a further sub-field - constituted by multiple hidden layers into the "neural" network - that does not necessarily require a labeled dataset.

Machine learning currently represents a powerful tool for several sub-fields of biology; the availability of good cyberinfrastructure and good training dataset is critical for utilizing machine learning at a full power in innovative ways and applied to different biological processes, while algorithmic development continues to evolve [8]. Since the interest in the study of biological interactions permeates system biology, there is the need of specialized repositories and advanced integration and visualization techniques; nevertheless, the utilization of such existing repositories makes the retrieval, combination, and manipulation of interaction evidence particularly difficult for inexperienced users [9]. The number and types of molecular interactions within repositories are rapidly growing and molecular networks are emerging as tools for understanding a variety of biochemical, statistical, and functional interactions; however, suitability of these networks for investigating a specific disease or pathway of interest still remains an open question [10].

All in all, bioinformatics (i.e., the discipline that applies information and statistical sciences for gathering and analyzing large sets of biological data) and computational biology (i.e., the discipline that aims to a better understanding of biological systems by taking advantage of data-based methods for biological simulations and modeling) offer scholars unprecedented (and often free) opportunities for 1) massively fastening literature research, 2) suggesting targets of investigation with a data-driven approach, 3) unveiling original insights, and 4) predicting possible scenarios. However, these amazing possibilities still need an epistemological criticism, as well as standardized procedures of investigation and modeling of novel study designs. The above-mentioned bioinformatic-assisted review is an example of how to walk these paths.

Virtually all research topics may benefit from DS-assisted methods, but especially those with a huge - otherwise said overwhelming - body of data. In this vein, since the early 2000s, skeletal muscle has received a growing interest both in physiology and pathology as the largest secretory system, with an ever-expanding body of evidence on muscle secretome which accounts for the cross-talk across systems and organs [11–15]. Myology is currently an appealing field of research attracting basic, translational, and clinical researchers [16], aware that the complexity of the muscle physiology and pathophysiology should be addressed using multidisciplinary approaches [17]. Hundreds of myokines - defined as peptides or proteins secreted or released from skeletal muscle cells exerting auto-, para-, or endocrine functions - have been described in the literature [13,18], so that bioinformatic approaches are needed for the analysis of a multicomponent communication network between skeletal muscle and other organs [19,20]. Computational analysis of muscle proteomics [20] and molecular approaches to depict muscle cell-specific secretome and trafficking [21] deepen the knowledge of myokines; establishing the molecular transducers of muscle adaptations [22] extend the knowledge to the inter-organ communication of muscle system; however, such studies requires a lot of time and large efforts. Therefore, computational predictions can be integrated with theoretical predictions prior to molecular and bioinformatic studies to fasten and deepen skeletal muscle research. Indeed, skeletal muscle secretome (otherwise called myokinome) represents an intriguing topic for applying DS tools and thereby defining a workflow possibly translatable into a plethora of other topics of interest, possibly as how aging or exercise or diseases affect the myokinome and vice versa. Thus, the emerging field of complex analysis might give new resounding and disruptive contributions to define and to evaluate this complex scenario. Our idea applied to the "myokinome" world is to present a possible combined expert-driven and data-driven approach for enlarging the use of bioinformatic tools by non-bioinformaticians in medicine and physiology.

1.1. Purposes

Within this background, this study used a DS-assisted and expert-supervised prediction and network analysis as a novel integrated procedure for fastening, deepening, and leading research on specific topics. In particular, this study looked at the world of myokines aiming to 1) criticize the possible role of AI tools for biomedical reviews and original studies, 2) provide novel targets of investigations, 3) show emerging insights through a network approach, and 4) provide unexpected targets of investigations by means of DS tools for biomedical review and network analysis as emerging powerful and integrated approaches.

2. Methods

2.1. Design of the study

The schematic workflow is presented in **Fig. 1**. Three recognized scholars with expertise on myokines were asked to provide a list (arbitrarily 10-to-50 items) of those myokines considered fundamental and/or the most interesting for human physiology. The three lists received were filtered in order to include only those proteins associated with a gene in humans; this inclusion criterion, as well as approved gene symbols, were checked into HUGO Gene Nomenclature Committee (HGNC, https://www.genenames.org/ [23]). The diverse backgrounds of the scholars resulted in remarkably different lists, although some commonalities were present, since almost all scholars agree on the importance of some of them. Moreover, this outcome allowed to proceed with the study flow. From the merged list, 4 out of 52 unique items were discarded as no associated gene exists - namely acetyl-L-carnitine,

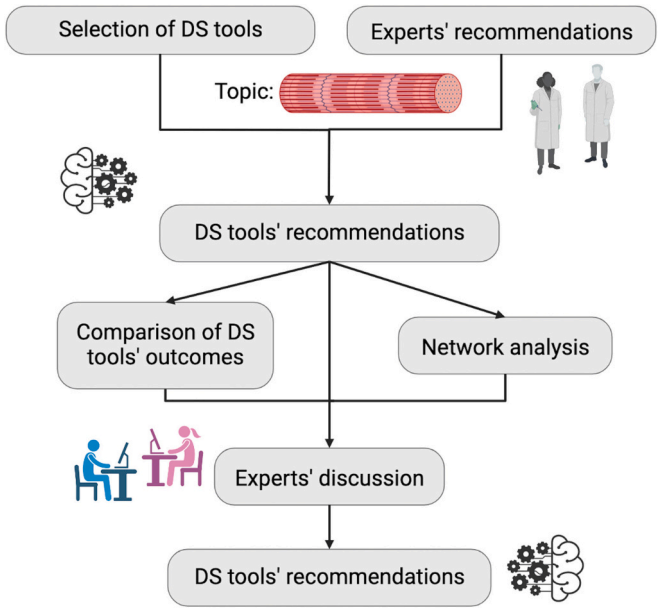

Fig. 1. Flow diagram of the study. Comparison of the AI recommendation was conducted in order to detect communalities; image created in Biorender.com

β-aminoisobutyric acid (BAIBA), nitric oxide (NO), and prostaglandin E2; - and 1 was discarded as labeled as not found in humans (amyrel), leaving a total of 47 myokines suggested.

Among the plethora of online bioinformatic tools, authors selected:

- GeneRecommender (GeneRecommender Platform - TheProphetAI - A.I. for Target Discovery, 2021; https://www.generecommender.com), due to the cooperative approach of the team linked to advanced AI approaches for recommending genes of interest;
- STRING (https://string-db.org), for the appreciated use in data analysis and visualization for protein-protein interactions [9,10,24]
- GeneMANIA (https://genemania.org) for the possibility of having gene recommendation, function prediction, and network visualization within the same tool [25]
- HumanNet (https://www.inetbio.org/humannet/) for the possibility of analyzing both gene and protein interaction networks within the same tool [26]

2.2. Experts' recommendations

The three experts' research topics encompasses molecular and cellular biology of striated muscles, myogenesis, muscle physiology and pathophysiology, cytokines and myokines, cancer cachexia, extracellular vesicles, and physical exercise. From the list of 47 myokines (hereafter *Model 1*), a sub-group of 19 consisted of items recommended by at least two out of three experts (hereafter *Model 2*), while a further sub-group of 5 consisted of items reported by all the 3 experts (hereafter *Model 3*). For a detailed description of experts' recommendations, see **Table 1**. The sets used as queries for DS tools are listed in **Supplementary Table 1.**

2.3. Procedures: data science tools

The platform GeneRecommender was used both with the current stable (I) and with the newly developed (II) algorithm. In this first analysis no disease was included as filter. The platform STRING was used with the multiple protein tool, either with or without text mining among the active interaction sources. The platform GeneMANIA was used with the online tool, rather than with the associated Cytoscape app. The platform HumanNet V.3 was used both with the protein - protein interaction (PI), functional (FN), and functional extended by co-citation (XC) network. The analysis was set choosing the network-based disease gene prediction options. Probability or likelihood of recommendations was evaluated by existing knowledge to depict the putative myokines.

2.4. In-depth box: features of data science tools

In this section the four platforms (GeneRecommender, STRING, GeneMania, and HumanNet) will be presented. The main goal is to provide the reader with all the most crucial elements to understand the peculiarities of each platform, their differences, and some elements about the algorithms that power them. Every platform will be presented in a separate paragraph.

The first platform here considered is the GeneRecommender, a deep learning-based platform that has been developed by TheProphetAI. It is designed to recommend some genes that could be related to given research. It works receiving, as input, a set of genes and/or a pathology. The algorithm behind the platform is a Deep Learning Neural Network, a subfield of AI, to predict the correlations between the output and the input gene set. The system is trained to propose new "correlations" that may not have an explicit reference within the literature. It is important to highlight that the training process of this algorithm, which characterizes all machine learning systems, has been performed on all publicly available literature in PubMed by applying advanced Natural Language Processing (NLP) methods to extract knowledge from papers. The team behind GeneRecommender, over the last two years, developed two versions of the core algorithms with increasing accuracy. The first version was released in July 2021, and the second in March 2022. Furthermore, even if the functioning of the system has not been released fully by the developers yet, the company provided shreds of evidence of the performance on established knowledge [27]. GeneRecommender, differently from other systems, uses Deep Learning techniques not only to extract knowledge but also to directly predict the proposed enrichment.

The second platform considered is STRING, a widely used protein-protein interaction investigation tool that gathers known and predicted data. The interactions that STRING present can be divided into two categories — direct (physical) and indirect (functional). As stated by the authors they stem from computational prediction, knowledge transfer among organisms, and interactions aggregated from other databases. In order to gather all these data STRING collects and assigns a score to pieces of evidence coming from different sources. Reporting from their paper [24]: (i) automated text mining of the scientific literature, (ii) databases of interaction experiments and annotated complexes/pathways, (iii) computational interaction predictions from co-expression and from conserved genomic context, and (iv) systematic transfers of interaction evidence from one organism to another. All these data are then aggregated and a pre-computed combined score is saved in their system. The combined score represents the level of confidence that STRING has about the mindfulness of a given association from a biological point of view.

GeneMANIA is a tool that screens for several gene relationships by looking at functional association input. These include protein and genetic interactions, pathways, co-expressions, co-localization as well as protein domain similarity. The user enters a list of genes and, optionally, selects from a list of data sets that he/she wishes to query. GeneMANIA then adds other genes that are functionally similar to the initial query genes and creates a functional interaction network of relationships all among the genes. The GeneMANIA algorithm is based on both an algorithm from ridge regression and a technique that computes composite functional association networks. Those two parts are fused within the GeneMANIA platform to create a unique system of analysis. Given the weights for each network, genes interact as much as possible with each other while interacting as little as possible with genes not on the list. A label propagation algorithm is the next step - it rates all non-query genes based on how often paths that start at them end up in one of the query

Table 1
The whole list of myokines of interest, created merging the lists provided independently by the three experts. Expertise of A lies on molecular and cellular biology of striated muscles, myogenesis and muscle pathologies. Expertise of B lies on muscle pathophysiology, myokines, and cancer. Expertise of C lies on extracellular vesicles, muscle signaling and physical exercise-induced cytokines.

Myokine	Gene	Expert	Event / pathology	Effect	Reference
Angiogenin	*ANG*	A	Pancreatic beta-cell/ diabetes	Protection	[119]
			Angiogenesis/cell growth and migration		[120]
				Positive effect	
ANGPL4	*ANGPL4*	B	Exercise / Fasting	Up	[121]
Apelin	*APLN*	A,B	Exercise	Up	[122]
			Cardiac failure	Positive	[123]
			Aging	Down	[124]
BDNF	*BDNF*	A,B,C	Diabetes	Down	[97]
			Nordic Walking training / Exercise	Positive effects on the brain	[99]
Cathepsin B	*CTSB*	A,C	Exercise / running	Upregulate BDNF / fitness and hippocampal memory function	[104]
Ciliary neurotrophic factor	*CNTF*	A	Neurodegeneration Weight loss (particularly fat mass)	Protection Positive effect	[125]
CXCL1	*CXCL1*	A	Antiproliferative and proapoptotic effects/ pancreatic cancer	Up	[47]
Decorin	*DCN*	A, B	Exercise / Physical functioning	Up / Positive correlation	[126]
FGF2	*FGF2*	A	Self-renewal of cancer stem cells / angiogenesis / poor prognosis in cancer	Up	[127] [128]
FGF21	*FGF21*	A,B,C	Sarcopenia/	Up	[129]
			Diabetes	Down/Up?	[130,131]
			Exercise / mitochondria	Positive and Negative effects / modulate cellular function and senescence	[100]
Follistatin	*FST*	A, C	Expressed in brown adipose tissue (BAT) and skeletal muscle	Promotes brown adipocytes-like functions in both white adipose tissue (WAT) and BAT	[105]
Fractalkine	*CX3CL1*	A,B	Diabetes	Up	[132]
FSTL1	*FSTL1*	A	Promotes growth and metastasis/ poor survival	Up	[133]
GBA	*GBA*	A	Risk of Parkinson's disease	Accumulation in brain (to be confirmed)	[134]
GDF11	*GDF11*	B	Aplastic anemia	Up	[135]
GDF15	*GDF15*	B	Fatigue syndrome	Positive correlation	[136]
HSP60	*HSPD1*	C	Exercise	Upregulation of PGC1α isoform α1	[101]
IGF-1	*IGF1*	A,B,C	Exercise/Diabetes	More effective control on glycemia	[97]
IL-10	*IL10*	A,C	Exercise/ Exercise induced bronchoconstriction	Up	[96]
IL-13	*IL13*	A	Repeated bouts of exercise	Up	[137]
IL-15	*IL15*	A,C	Exercise/Muscle anabolism	Upregulated	[93,94]
IL-1ra	*IL1R1*	A	Exercise	Anti-inflammatory environment	[138]
IL-3	*IL3*	A	Aerobic exercise in elderly	Lower in plasma	[139]
IL-4	*IL4*	A	Exercise	Increase insulin sensitivity	[140]
IL-6	*IL6*	A,C	Exercise	Positive effects	[91,102]
IL-7	*IL7*	A	Resistance training in overweight women	Down	[141]
IL-8	*CXCL8*	A,C	Exercise / Bronchoconstriction	Up	[96]
Insulin-like 6	*INSL6*	A	Muscle injury	Stimulate myogenic regeneration	[142]
Irisin	*FNDC5*	A,B,C	Exercise	Up/ increases cell proliferation Up	[98]
			Diabetes	Up	[97]
			Exercise in COPD*	Mostly reduced in serum, upregulated in cancer tissue	[143]
			Cancer		[144]
Leukemia inhibitory factor	*LIF*	A,C	Dystrophy/acute training	Role in normal muscle biology	[92]
Meteorin-like	*METRNL*	A,C	Induced in muscle after exercise and in adipose tissue upon cold exposure	Improves glucose tolerance and stimulate beige fat thermogenesis and anti-inflammatory cytokines	[106]
Musclin (osteocrin)	*OSTN*	A,B,C	Diabetes	Up	[145]
			Burn injury	Up	[146]
			Fast-glycolytic phenotype	Enhances physical endurance by promoting mitochondrial biogenesis	[147]
Myonectin (CTRP5) **	*C1QTNF5*	A,B	Diabetes	Marker, positive correlation	[148]
Myostatin (GDF8)	*MSTN*	A,C	Burn injury	Up	[149]
			Developing and adult skeletal muscle	Negative regulator	[150]
			Endurance and resistance exercise		
				Down	[151]
NGF (nerve growth factor)	*NGF*	A	Reduced *in vitro* and *in vivo* nociception	R100W mutation in *NGF*	[152]
Neurotrophin-4	*NTF4*	A	Neuromuscular connections and performance	Positive effect	[153]
Oncostatin M	*OSM*	A	Induction of chronic inflammation, vascular injury and fibrosis	Up	[113]
Osteoprotegerin	*TNFRSF11B*	A	Endothelial dysfunction in metabolic disorders	Positive association	[154,155]
			Marathon (72 h after)		
				Up	[156]
RANTES	*CCL5*	B	Cardiac mortality	Down	[157]
S100A8	*S100A8*	A	Exercise	Inflammatory response	[158,159]

(*continued on next page*)

Table 1 (*continued*)

Myokine	Gene	Expert	Event / pathology	Effect	Reference
S100A9	*S100A9*	A	Exercise	Inflammatory response	[158,159]
S100B	*S100B*	A	Exercise	Brain damage and blood-brain barrier disruption	[160]
SDF-1	*CXCL12*	B	Age / osteoporosis / sarcopenia / cachexia	Down	[161,162]
SPARC	*SPARC*	A,B	Exercise	Up	[163]
			Cancer	Less risk of death	[164]
TNF-α	*TNF*	A	Inflammation and cancer progression	Up	[165]
					[166]
VEGF	*VEGFA*	C	Exercise	Pro-angiogenic	[167]
Visfatin	*NAMPT*	A	Cardio-metabolic diseases	Positive association	[168,169]

Note: Acetyl-L carnitine, BAIBA, NO, and PTGE2 were reported but discarded from further analyses, since they are not protein products associated with a gene; amyrel was suggested but discarded, since not found in humans. *Chronic obstructive pulmonary disease; ** one expert considered CTRP5, while the other CTRP15, as myonectin; there is a mismatch in literature on this topic; actually, the myokine myonectin should be considered as C1QTNF15, or CTRP15, codified by Erythroferrone (ERFE) gene; however, a large body of research focused on CTRP5, and looking at myonectin in GeneCards, the highest relevance score was achieved by CTRP5, ahead of ERFE, and the first one was therefore considered in our research

nodes and how long and heavily weighted those paths are before they do.

Finally, HumanNet is a large-scale, human gene functional interaction network incorporating diverse expressions, protein interactions, genetic interactions, sequences, literature, and comparative genomics data. The network includes both data collected directly from human genes, as well as that from orthologous genes of yeast, worm, and fly. In total, 21 large-scale genomics and proteomics data sets from the four species were integrated, spanning 476,399 scored functional couplings between 16,243 (87%) of validated human protein encoding genes. Given a set of known related genes, additional genes can be predicted by their weighted associations in the network, with more strongly connected genes being prioritized more highly. This is achieved using standard label propagation algorithms, like naive Bayes GBA, or more advanced algorithms related to Google's PageRank, like iterative ranking and gaussian smoothing. Example applications include the identification of target genes in diseases studies: knowing a few genes implicated in a disease, the network offers a strong tool for prioritizing additional likely candidate genes. HumanNet allows to select among three reference networks: PI (protein-protein interaction network), FN (functional gene network), and XC (FN extended by co-citation).

2.5. Procedures: data analysis

The outcomes of DS tools included arbitrarily the top 5 recommendations for each tool, selecting Homo Sapiens as the target species/organism. Such outcomes were qualitatively and quantitatively compared. For this comparison, each tool was considered as a unique system (i.e., if GeneRecommender I and II suggested the same molecule, this was considered only once; if HumanNet PI, FN, and XC suggested the same molecule, this was considered only once; if STRING TM and NoTM suggested the same molecule, this was considered only once). Firstly, the presence of commonalities across the outcomes was checked. Then, all the suggestions were checked for their possible interpretations as myokines. Particularly, the outputs were described using The Human Protein Atlas (http://www.proteinatlas.org), which enables us to look at the human secretome, including those proteins retained intracellularly [28]. Tissue specificity, extracellular location, molecular function, and disease involvement were collected, eventually integrating missing data with UniProt (https://www.uniprot.org/), which constitutes an ever-updating resource of protein sequence and functional information [29].

Given that extracellular vesicles (EVs) successfully deliver myokines from muscle cells to other organs [30,31], even though skeletal muscle-released EVs may only account to a subtle extent on circulating EVs [32,33], the established and candidate myokines as recommended by DS tools were searched on Vesiclepedia (http://www.microvesicles.org). This tool is a compendium of molecular data identified in different classes of EVs and still represents a continuous community annotation project, where EVs researchers are actively engaged with direct data sharing [34]. For this analysis, only Organism=Homo Sapiens and Identified molecule=protein were considered, and biological samples were annotated.

Network of associations were built as follows:

- in STRING the setting consisted of experiments, databases, and co-expression, with medium confidence of 0.400 and no more than 5 interactors, full network, including, false discovery rate (FDR) < 5%; the Markov clustering (MCL) algorithm was used [35] and protein - protein interactions (PPI) enrichment p-value, average node degree, and local clustering coefficient were calculated;
- in GeneMANIA, the setting consisted of physical interactions, co-expression and genetic interactions, with no more than 5 resulting genes, molecular function- based gene ontology (GO) weighting and FDR < 5% for mapping functions.

The secondary DS-based analysis after the discussions among experts was carried out as follows:

- in GeneRecommender the input genes were analyzed through both DeepProphet 1 and DeepProphet 2 algorithm, eventually selecting the diseases as discussed into the experts' in-depths;
- in HumanNet the same input genes were analyzed through the XC network-based disease gene prediction.

3. Results

The reports of the experts presented as expected communalities but were also rather heterogeneous both in quantity of items and topics. To provide info for translational perspectives, the possible disease involvement of each molecule was reported. As shown in **Supplementary table 2**, the most represented disease was cancer; concerning molecular functions, the signaling of cytokines and growth factors were the most represented. Greater heterogeneity was found in tissue specificity.

The list of the top five recommendations in shown in **Table 2**. The resulting recommendations were prioritized by the tools basing on their scoring algorithms and can be interpreted as the main nodes into a virtual interactome. For what concerns commonalities across the tools' recommendations:

- Model 1 ranked ***CCL2*** and ***IL1B*** as first, with 2 recommendations each, both from GeneRecommender (IL1B from version I and II, CCL2 only from version II) and HumanNet (XC);
- Model 2 ranked ***IL10RA*** (STRING TM and NoTM, GeneMANIA), ***IL6ST*** (HumanNet PI, STRING NoTM), ***SORT1*** (HumanNet PI, STRING TM), and ***TFGB1*** (HumanNet FN and XC, GeneRecommender II) as first, with 2 recommendations each;
- Model 3 ranked ***IGF1R*** (Human Net PI and FN, GeneMANIA, STRING TM and NoTM) and ***NTF4*** (Human Net PI and FN, GeneMANIA,

Table 2
The comprehensive list of the top five recommendation by each tools; the recommendations are ordered by the highest to the lowest score, as attributed by each tool

Input selection model	Gene Recommender		HumanNet V3			Gene Mania	STRING	
	*I**	*II***	*PI*°	*FN*°°	*XC*°°°		*TM*^	*NoTM*^^
Reported by at least 1 expert (n=47)	INS	TGFB1	A2M	CCL20	IL1B	NTF3	TNFRSF1A	IL10RA
	IL1B	INS	CCR1	INHBA	CCL2	IL21	IL10RA	KDR
	IL1A	IL1B	SORT1	CCL3	CXCL5	IL9	IL2RB	TNFRSF1A
	TGFB1	CCL2	IL6ST	CXCL9	CCL20	IL20	NGFR	TNFRSF1B
	LEP	IL1A	IL2RG	GRB2	CCL3	IL19	IGFBP5	HSPE1
Reported by at least 2 experts (n=19)	INS	INS	AM2	INHBA	CD14	CSF3	IL10RA	IL6R
	LEP	ADIPOQ	IL6ST	GRB2	TGFB1	OSM	IL2RB	IL6ST
	TNF	TNF	SGTA	PRKACA	CCL3	IGF2	NGFR	IL10RA
	ADIPOQ	LEP	SORT1	TGFB1	CXCL1	KLB	SORT1	IGF1R
	VEGFA	TGFB1	COL14A1	SOS1	IL1B	IL10RA	IGFBP5	CXCR2
Reported by all the 3 experts (n=5)	INS	INS	IGFBP3	TNF	FGF2	NTF4	IGF1R	IGF1R
	LEP	MSTN	NTF4	IGF1R	PGC	IGF1R	NGFR	INSR
	GH1	FOXO1	IGFBP4	NTF4	AGRP	IGFBP6	IGFBP5	NTF3
	IL6	LEP	IGF1R	NTRK2	UCP1	NTF3	IGFBP4	NTF4
	MSTN	IGF2	KLB	SOS1	SIRT1	IGFBP5	SORT1	IGFBP4

*previous algorithm; ** current algorithm; °protein-protein interaction network; °°functional gene network; °°° functional gene network extended by co-citation; ^ including text mining in research filters; ^^ not including text mining in research filters

STRING NoTM) as first, with 3 recommendations each, and ***IGFBP4*** (Human Net PI, STRING TM and NoTM), ***IGFBP5*** (GeneMANIA and STRING TM), and ***NTF3*** (GeneMANIA and STRING NoTM) as second, with 2 recommendations each

Among the 62 unique outcomes from DS tools' recommendations, as shown **in** Fig. 2:

- 8 molecules had already been suggested by the experts (it needs reminding that Model 2 and 3 included 19 and 5 myokines, respectively, leaving the possibility that the recommendations would result in myokines included in the whole list of 47 myokines as in Model 1)
- 11 molecules had already been described as bona-fide myokines in literature
- 11 molecules were putative myokines (considering the molecule type, gene expression and/or localization in muscle tissue, similarities of function with other myokines, presence in the bloodstream, mechanism of action)
- 32 molecules were still unlikely to be demonstrated as myokines

This clustering was used to quantify the number of recommendations that are certainly or possibly myokines, out of the top 5 indicated by each AI tool.

Most of the myokines and the putative myokines recommended by the DS tools were described as present in EVs cargo, as shown in Table 3.

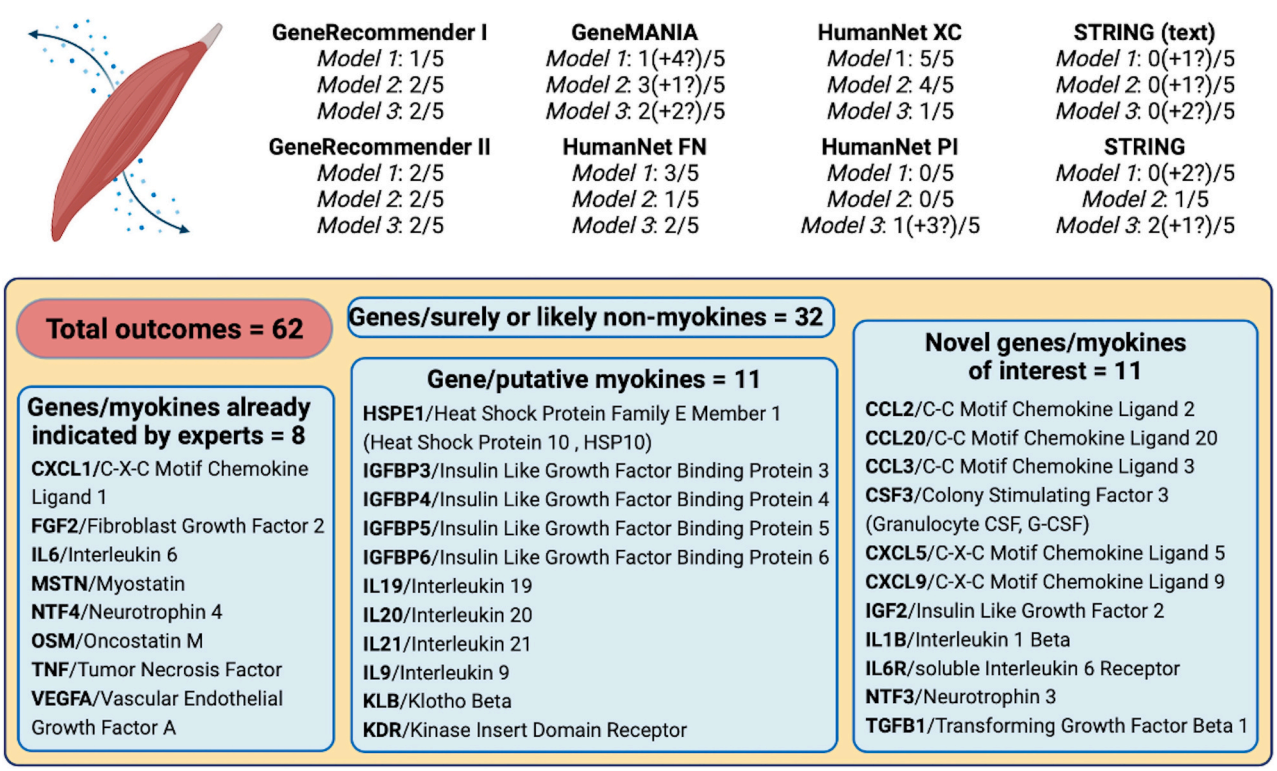

Fig. 2. Outcomes of AI tools; in the upper part of the image, x(+y?) refers to the number of certain (+ possible) myokines/genes out of the top 5 recommendations indicated by the tools; image created in Biorender.com

Table 3
Report of AI recommended and putative myokines if stuffed into EVs

Myokines	Vesicle type	Sample
CCL2	Exosomes	Bone marrow mesenchymal stromal cells, leukemia cells, T cells
CCL20	EVs/ exosomes/ microvesicles	Brain cancer cells, breast cancer cells, colorectal cancer cells, kidney cancer cells, leukemia cells, melanoma cells, mesenchymal stem cells, ovarian cancer cells, **normal urine**
CCL3	Exosomes	Serum -tuberculosis patient
CSF3	Exosomes	Mesenchymal stem cells
CXCL1	EVs/ exosomes	Brain cancer cells, breast cancer cells, colorectal cancer cells, leukemia cells, lung cancer cells, melanoma cells, ovarian cancer cells
CXCL5	EVs/ exosomes	Brain cancer cells, breast cancer cells, colorectal cancer cells, kidney cancer cells, leukemia cells, lung cancer cells, melanoma cells, ovarian cancer cells, prostate cancer cells, retinal pigment epithelial cells
CXCL9	N.A.	N.A.
IGF2	EVs/ exosomes/ membrane vesicles	Brain cancer cells, breast cancer cells, colorectal cancer cells, leukemia cells, lung cancer cells, melanoma cells, ovarian cancer cells, prostate cancer cells, **normal urine**
IL1B	N.A.	N.A.
IL6R	N.A.	N.A.
NTF3	Exosomes	Placental mesenchymal stem cells
TGFB1	EVs/ exosomes/ microvesicles/ microparticles	Brain cancer cells, breast cancer cells, colorectal cancer cells, embryonic kidney cells, glioblastoma cells, kidney cancer cells, leukemia cells, mesenchymal stem cells, ovarian cancer cells, **normal plasma**, blood of patients undergoing external counterpulsation therapy, platelets of normal donors, retinal pigment epithelial cells, serum by brain tumors, umbilical cord mesenchymal stem cells
Putative myokines		
HSPE1	EVs/ exosomes/ microvesicles/ ectosomes/ microparticles/ membrane vesicles/ membrane blebs/ nanovesicles/ apoptotic bodies	Astrocytoma cells, B cells, breast cancer cells, chronic lymphocytic leukemia cells, colorectal cancer cells, dendritic cells, endothelial cells, epithelial cells, glioblastoma cells, kidney cancer cells, leukemia cells, lung cancer cells, melanoma cells, neonatal myoblast cells, ovarian cancer cells, placenta, **plasma**, platelets, prostate cancer cells, retinal pigment epithelial cells, squamous carcinoma cells, T cells, umbilical cord mesenchymal stem cells, **normal urine**
IGFBP3	EVs/ exosomes/ membrane vesicles/ microvesicles	Brain cancer cells, breast cancer cells, normal breast milk, colorectal cancer cells, kidney cancer cells, leukemia cells, lung cancer cells, melanoma cells, mesenchymal stem cells, ovarian cancer cells, prostate cancer cells, serum by tuberculosis patients, **normal urine**
IGFBP4	EVS/ exosomes	Brain cancer cells, breast cancer cells, colorectal cancer cells, kidney cancer cells, leukemia cells, lung cancer cells, melanoma cells, mesenchymal stem cells, ovarian cancer cells, prostate cancer cells, serum by tuberculosis patients
IGFBP5	EVs/ microvesicles	Brain cancer cells, breast cancer cells, colorectal cancer cells, kidney cancer cells, leukemia cells, lung cancer cells, melanoma cells, ovarian cancer cells, umbilical cord mesenchymal stem cells
IGFBP6	EVs/ exosomes/ membrane vesicles	Aqueous humor, brain cancer cells, breast cancer cells, colorectal cancer cells, kidney cancer cells, leukemia cells, lung cancer cells, melanoma cells, mesenchymal stem cells, ovarian cancer cells, prostate cancer cells, serum by tuberculosis patients, **normal urine**
IL19	Exosomes	Bovine milk, breast milk, mesenchymal stem cells
IL20	N.A.	N.A.
IL21	N.A.	N.A.
IL9	Exosomes	Serum by tuberculosis patients
KDR	N.A.	N.A.
KLB	N.A.	N.A.

Note: EVs= Extracellular vesicles; only Organism=Homo Sapiens and Identified molecule=protein were considered

In addition to the findings in pathological samples, HSPE1, IGFBP3, IGFBP6, CCL20 and IGF2 were reported in urine and both HSPE1 and TFGB1 in plasma. While several results demonstrated the presence of CCL, CXCL, and IGFBP families, as well as HSPE1, in a plethora of tissues, interleukins are yet to be fully explored as transferred by EVs.

Network analysis of the recommendations, whose results are shown in **Fig. 3**, was performed both on STRING and GeneMANIA. The MCL algorithm implemented into STRING resulted in 6, 3, and 2 clusters for Model 1, 2, and 3, respectively; Model 1 was built with 75 edges, model 2 with 28, and model 3 with 14; nodes were all shown. GeneMANIA algorithm in all the three models resulted in a highly disconnected node, namely OSTN; the most important functions mapped, among the others, were related to cytokines and muscle regenerative pathways in Model 1 and Model 2, while Model 3 was functionally related to growth signaling.

4. Discussion

Currently, the big data problem is facing biomedical sciences with ever-expanding, extremely large volumes of both raw data and scientific articles. Therefore, DS has been pervading almost all aspects of the scientific world, in order to assist scholars in analyzing large datasets and retrieving the core info from literature. As for the latest topic, for example, scholars can be helped by distinguishing between relevant and irrelevant literature on subtle differences identified through text mining tools [36], as well as performing bioinformatic assisted reviews [4].

It can be claimed the need for integrating human and artificial intelligence [37] for advancing biomedical research. Here, coping with a scientific topic currently representing a fertile area of research [13] and starting from experts' recommendations, we identified established and potential myokines linked to the input myokines, using a supervised DS approach.

4.1. Comment on the usage

As expected, the chosen DS tools provided diverse recommendations, due to the diverse characteristics of research algorithms. All the tools exhibited good usability, although the selection of research filters requires specific knowledge. The field of bioinformatic tools is greatly fertile, and during the writing of this work novel advancements have been made.

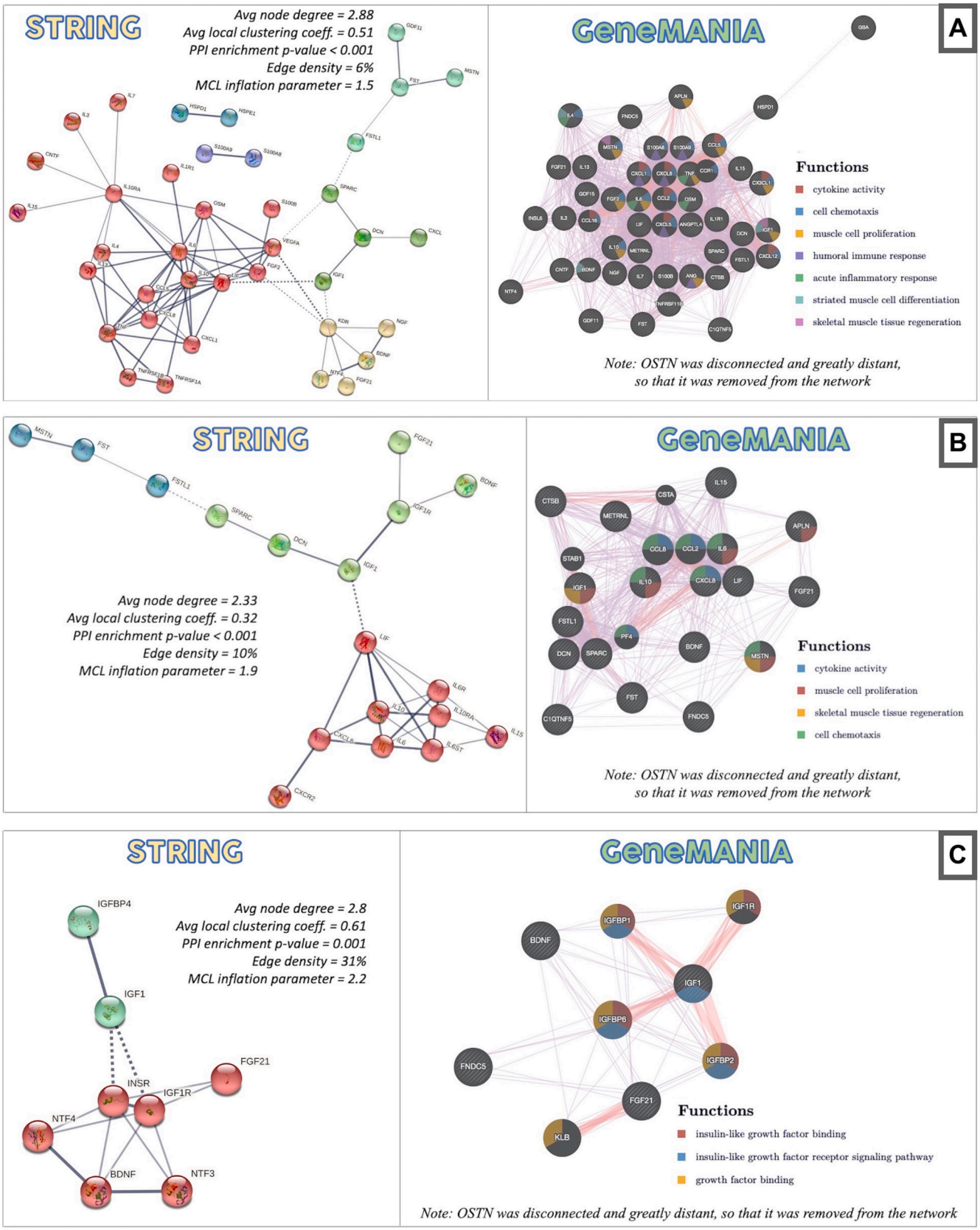

Fig. 3. Graphical results of network analysis conducted on STRING and GeneMANIA on both Model 1 (A), Model 2 (B), and Model 3 (C). The lists used as queries can be retrieved from **Supplementary Table 1.**

In order to spread the usage and the effectiveness of DS tools in biomedical research, a possible approach may be a first user-friendly interface with basic features, followed eventually by an advanced interface for users capable of selecting special features. An appreciated characteristic of DS tools is both the analytical integration and the link with other DS tools.

Comprehensive state-of-the-art suggestions of DS tools, as well as standardized procedures for integrating them into the biomedical workflow are needed. Similarly, methods for comparing results and guidelines for selecting the features would be helpful. Finally, graphic tools for organizing the outcomes and disseminating the insights would be greatly appreciated by researchers.

4.2. Experts' discussion

Experts were required to select an original topic, integrating their knowledge with the DS tools' recommendations, thus providing original insights. This efforts serve as examples of how to use prioritizations of molecules of interest from DS tools' algorithms (in this case the top 5 recommendations) for creating novel biological interpretations and hypotheses to be furtherly investigated. As a result, the following boxes represent the in-depths of experts, based on reported inputs and outputs, on a topic of their expertise and interest. Each expert-led discussion has been consequently integrated with an DS-led approach to provide additional perspectives.

4.3. In-depth box 1: The role of myokines in PDAC - a focus on FGF21 and IL6

Some of the reported myokines exert different roles in several cancer types, including pancreatic ductal adenocarcinoma (PDAC) [38]. PDAC is the most common and aggressive type of pancreatic cancer with a 5-year relative survival rate lower than 10%, mostly contributed by late diagnosis and limited efficacy of current systematic treatments [39].

The role of physical activity in reducing the risk of PDAC is well-established [40–42]. The release of myokines in response to muscle contraction might, at least in part, explain the anti-tumor effect [43]. Among hundreds of myokines, only few have been clearly correlated with anti-proliferative effects in PDAC, in particular irisin. It was shown: 1) to reduce pancreatic cancer cell growth via AMPK/mTOR signaling and affect the invasion and migration of pancreatic cancer cells through inhibition of epithelial-mesenchymal transition [44]; 2) to positively regulate ferroptosis (a particular form of regulated cell death) in Panc1 (a human pancreatic cancer cell line), thus increasing ROS-mediated processes and autophagy [45]; 3) to improve doxorubicin-induced cell apoptosis in pancreatic cancer cells (MIA PaCa-2 and BxPC-3) by inhibiting the PI3K/AKT/NF-kB pathway [46].

Interestingly, a recent study found three other myokines with anti-tumor effects in PDAC [47]. The authors discovered an increase in protein and mRNA levels of IL10, CXCL1 and CCL4 in exercise-conditioned serum derived from advanced PDAC patients after 12-week of resistance training using whole-body electro-myostimulation. The administration of recombinant human IL10, CXCL1 and CCL4, particularly in combination, exhibited strong anti-proliferative and anti-migration effects in Panc1 cells, which was associated with increased mRNA levels of CASP3/7 and DNA fragmentation. Of note, the same effects could not be observed when non-malignant pancreatic cells were exposed to the recombinant proteins. However, these results are observed in the 2D model and thus it would be interesting to investigate these beneficial functions also with a different approach, such as the patient-derived organoids (3D model) that is able to recapitulate all stages of the disease and the genomic and transcriptomic landscape of PDAC [48,49].

Moreover, the main component of the tumor microenvironment (TME), the cancer-associated fibroblasts (CAFs), deserves attention. Indeed, CAFs play crucial and opposing functions in defining the biology and the aggressive phenotypes of PDAC [50–53]. Recently, an important role of fibroblast growth factor 21 (FGF21) was identified in pancreatic tumorigenesis. Both in mice and humans, normal acinar cells show high levels of FGF21. Loss of acinar differentiation and ductal metaplasia is a key event during tumorigenesis of the pancreas in mice and it is driven by the oncogenic activation of Kras [54]. Accordingly, activation of Kras in pancreatic exocrine cells reduces the expression of FGF21 and treatment of mice with recombinant human FGF21 (rhFGF21) reduces pancreatic tissue inflammation and delays neoplastic progression [55]. Interestingly, the beneficial results were obtained also in the presence of a high-fat diet (which strongly increases the risk to develop PDAC) [55].

High circulating levels of IL6 are associated with cachexia [56] that consists of progressive and debilitating loss of body mass with muscle depletion and a worse prognosis [57] in PDAC. Cachexia affects about 90% of patients with PDAC [58] and was recently found in the early phase of PDAC [59]. It is worth recalling that the prototypical myokine IL6 has shown a strong proinflammatory function when released by immune cells but IL6 exerts anti-inflammatory action when released by the muscle [43]. Beyond the discovered crosstalk regarding IL6 in promoting cancer proliferation and immune suppression in TME of PDAC [50], new tumor-tissue crosstalk mediated by IL6 and soluble IL6 receptor (sIL6R) trans-signaling was identified: IL6 released by tumor cells triggers a positive loop of IL6 release between fat (the major source of IL6) and skeletal muscle (the major source of circulating sIL6R that accumulates in the fat), thereby promoting cachexia and cancer progression [60].

4.4. In-depth box 2: Myogenesis, regeneration and brain function - a focus on S100B

S100B is an EF-hand type calcium-binding protein with intracellular and extracellular activities [61]. It is expressed by several cell types of nervous and non-nervous origin, with astrocytes expressing the highest amount of the protein. At muscle level, S100B is expressed by satellite cells and their progeny (i.e., the myoblasts and the myotubes obtained by their fusion) and myofibers [62,63]. In myoblasts, intracellular S100B regulates myogenic differentiation, with high levels inhibiting it via NF-kB-dependent inhibition of MyoD expression, and S100B regulates cell proliferation, and the transition from proliferation to quiescence and vice versa [64,65]. Behaving as a damage-associated molecular pattern (DAMP), S100B is released from muscles early upon acute injury and is required for a rapid and complete regenerative process, favoring the expansion of muscle precursor cells, attracting macrophages and promoting their polarization into the M2 phenotype, and modulating the collagen deposition, by interacting with receptor for advanced glycation end-products (RAGE) or fibroblast growth factor receptor 1 (FGFR1) [66,67]. In the central nervous system, low concentrations of S100B exert neurotrophic effects, induce neurite outgrowth and promote neuron survival [68]. S100B modulates neuronal synaptic plasticity, learning and memory [69], and promotes neurogenesis in the hippocampus [70]. Since S100B serum levels increase after intense physical activities [71], S100B might concur to the cognitive improvement associated with physical exercise.

Interestingly, S100B has been reported to interact with basic fibroblast growth factor (bFGF/FGF2) thus enhancing FGFR1 signaling and blocking the S100B canonical receptor, RAGE to stimulate proliferation and inhibit differentiation in high-density myoblasts [66,72]. A similar engagement of FGF2 was observed in low-density myoblasts in the presence of relatively high doses of S100B so that the proliferation of satellite cell-derived myoblasts subsequent to muscle injury is mainly dependent on the bFGF-mediated activation of FGFR1 by S100B [66]. Structural models of S100B-FGF2 complex were generated, and the critical residues on S100B-FGF2 interface were mapped by NMR spectroscopy and site directed mutagenesis [73].

Among the novel potential myokines emerged by AI-based recommendations is CCL2, which appears also in the network of S100B

interacting proteins (Fig. 3). Interestingly, i) a positive correlation was found between S100B and CCL2 expressions in human proneural and neural glioma subtypes, and ii) S100B has been reported as an important inducer of CCL2 in gliomas promoting the recruitment of tumor-associated macrophages and tumor growth in vivo [74]. In accordance, blockade of S100B with a specific antibody caused a reduction of the levels of CCR2 and CCL2 in injured muscles suggesting a role for S100B in macrophage migration towards damage sites [67]. Of note, the mobilization of monocytes/macrophages from blood to acute damaged muscles and the muscle regeneration process were similarly impaired in Ccl2-/- and anti-S100B-treated mice [67,75].

4.5. In-depth box 3: tumor-to-muscle and muscle-to-tumor cross talks

Over the last years, it has been becoming clear that tumor and muscle crosstalk to each other in both directions. Tumors have been recognized as able to release factors enwrapped or not in EVs that are able to cause muscle wasting, during cancer cachexia. Some examples of tumor-derived factors that travel in the bloodstream by means of vesicles are Hsp70 and 90 [76] as well as certain miRNAs as miR-21 [77].

On the other way around, muscles have been recognized as secretory tissues able to release factors known as myokines that may or not communicate with tumors. Some examples are irisin [78], SPARC [79] and FGF21 [80]. Most myokines travel through the body protected by the bilayers of vesicles but it is still largely unknown how they target the tumors and/or metastases [31]. Notably, the release of vesicles and muscle contractions are both under the control of calcium-based signaling such to explain why most of myokines-enriched vesicles are inducible by physical exercise. It would be interesting to apply the tools described herein to analyze separately the myokinome in healthy and diseased conditions and see if and how it can respond to exercise, by expanding the knowledge on myokines reports [12]. These studies shall be complemented by analyses on the expression of the ligand (myokine) and its receptor (when it has been identified) both in muscles and tumors, in the so-called ligand-receptor prediction studies, for which the contribution of bioinformaticians will be crucial as well.

Regardless the tumor type, the expression (and the release in the blood) of some myokines are aberrantly affected during muscle wasting during cancer [12]. Myostatin is a myokine generally less released during exercise and more released by muscles under disease conditions, including cancer cachexia [81–83]. Myostatin is a negative regulator of muscle size [84] and its role on tumor growth shall be analyzed more in depth, because of conflicting reports in this regard [85,86].

Another example is musclin or osteocrin that resulted through multiple analyses herein shown apart from all the other myokines analyzed for reasons that surely deserve further investigations. Musclin is a myokine that we found reduced in the muscle and blood of mouse bearing various cancer types (sarcoma, lung carcinoma and C26 colon adenocarcinoma) even before the muscles were reduced in size (i.e., cachexia has started) [87]. Musclin drop in muscle and plasma at this early time makes it a suitable early biomarker of cancer cachexia at least in rodent models. Notably, physical exercise is sufficient to increase musclin in plasma and muscles [88] and in mice with cancer cachexia aerobic exercise but not anaerobic one is able to restore normal expression and plasma levels of musclin even in cancer-bearing mice [87]. Overall, this study supports the notion that the beneficial effects of exercise can still work even at molecular levels in those animals being affected by tumors.

The receptor of musclin is Npr3 or natriuretic peptide receptor 3 and its expression is also decreased in muscles under cachexia but not in a way that increasing locally musclin by means of muscle electroporation of plasmids may not obviate myofiber shrinkage induced by C26 growth. It would be interesting (i) to test if the same occurs also in the blood and muscles of cancer patients exhibiting or not cachexia; (ii) to understand how musclin interacts or not with other myokines of biological interest responsive to exercise (as myostatin, SPARC, SDF1/CXCL12 or apelin), (iii) to learn whether musclin is released through vesicles or not, and (iv) if we can manipulate musclin expression and release through miRNA or drugs or nutraceuticals. This last issue (iv) is crucial to solve because not all cancer patients are still in the conditions to complete periodic exercise sessions that may give them advantages for preserving their health, so for them an exercise-independent way to increase musclin would be helpful.

4.6. In-depth box 4: the role of myokines on skeletal muscle in response to physical exercise

Myokines have a physiological role on the skeletal muscle in response to physical exercise. Between the class of interleukins (ILs), muscle contractions mediated by physical exercise provoke IL-6, IL-8, IL-10, and IL-15 release to modulate systemic inflammation and lead chemotactic responses for muscle regeneration [89,90]. The circulating IL-6 protein expression after a single bout of exercise stimulates the glucose metabolism improving the expression of the insulin-regulated glucose transporter 4 (GLUT4) in soleus and plantaris muscles [91]. Between the IL-6 family members, leukemia inhibitory factor reveals to have significant muscle regeneration properties for the exercise injuries and for the dystrophic pathologies [92].

A significant anabolic effect of skeletal muscle has been established for IL-15 that induces myosin heavy chain hypertrophy in differentiated primary human skeletal myogenic culture, conversely to IGF-I that stimulates the myogenic precursor cells [93]. Besides, in vivo, a single bout of eccentric and concentric muscle contractions results in a significant increase of IL-15 mRNA level in the vastus lateralis muscle [94] and in the circulation to induce pro-oxidative mediators [95]. In addition, other circulating myokines/interleukins such as IL-8 and IL-10 are improved during exercise performance in humans, with regard to male marathon runners presenting exercise-induced bronchoconstriction to increase oxygen delivery to the muscles [96]. Considering the results of the gene recommendations, specific studies may be tailored to investigate the response in IL-1B, IL-9, IL-19, IL-20, IL-21 triggered by physical exercise, since these dynamics are currently unknown.

Contracting skeletal muscles also induces the systemic release of specific myokines that control the neuromuscular performance [97] like irisin that promotes hippocampal neurogenesis, mitochondrial biogenesis, and glucose homeostasis [98,99], BDNF for supporting CNS plasticity [99], and IGF-1 [93,97]. Exercise-induced adaptive changes include also FGF21 that is an insulin-regulated myokine. FGF21 physiologically regulates glucose-lipid metabolism and pathologically induces mitochondrial dysfunction with a bioenergetically detrimental effect on growing muscles [100]. Otherwise, beneficial mitochondrial action is mediated by Hsp60, whose mRNA expression is improved after a single acute bout of endurance exercise. Hsp60 protein expression is fiber I-type specific in the mice posterior group of hindlimb muscles (gastrocnemius, soleus, and plantaris) after endurance training of 6 weeks. This finding is in correlation with higher mitochondrial copy number and expression of the dominant regulator of oxidative metabolism, PGC1α [101,102]. This physical exercise-mediated improved mitochondrial function is also associated with the L isoform of BAIBA that works as a neuroprotector factor against oxidative stress activating the AMPK and PI3K/Akt pathways [103].

Myokine cognitive benefits have also been proved for Cathepsin B in adult male mice. Specifically, running elevates Cathepsin B in the plasma and the gastrocnemius muscle in order to cross the blood brain barrier and to enhance brain functions such as hippocampus-dependent memory and adult neurogenesis [104].

To regulate energy homeostasis, follistatin has been proposed as a regulator of both classical brown adipocyte precursors and an inducer of p38 MAPK/ERK signaling pathway to promote brown adipose expansion and activity [105]. Otherwise, meteorin accelerates energy expenditure through fat thermogenesis [106].

Among the list of novel and putative myokines, the complex

signaling regulated by IGF binding protein family is worth exploring. Also IGF2 deserves a special attention, as several polymorphisms of this gene are associated with muscle strength, and IGF2 is involved in exercise-induced muscle damage [107].

4.7. Data science tool secondary recommendations

All in all, the in-depth box number 1 on muscle-pancreas cross-talk which account for pancreatic cancer and cachexia is based on 5 myokines present in the experts' recommendation, 1 DS -recommended myokine (sIL6R) and 1 additional myokine (CCL4). Using the related 7 genes as input, in addition with "carcinoma, pancreatic ductal" (MeSH Unique ID: D021441) and "cachexia" (MeSH Unique ID: D002100) as diseases, GeneRecommender's DeepProphet 1 (former Genes Disease V.1) algorithm reported TNF as main gene of interest, followed by insulin, CRP and interleukins, while GeneRecommender's DeepProphet 2 algorithm pointed out, in addition to TNF, the possible involvement of CRP, CXCL8, and adipokines. The HumanNet-XC algorithm confirmed the main association with TNF, followed by IL1B and IL1A. Indeed, TNF-α has been reported as a key player on the molecular pathways of cancer cachexia, with both catabolic induction and anabolic inhibition [108], since decades ago [109]. Interestingly, TNF was included into the first experts' recommendation list.

The in-depth box number 2 is based on two myokines present in the experts' recommendations (S100B and FGF2), 1 DS-recommended myokine (CCL2) and 2 additional proteins (FGFR1 and RAGE). Using the related 5 genes as input (RAGE is an alias standing for AGER), in addition with "inflammation" (MeSH Unique ID: D007249), GeneRecommender's DeepProphet 1 algorithm reported IL6 as main gene of interest, followed by and TNF, while GeneRecommender's DeepProphet 2 algorithm pointed out VEGFA as main gene of interest, followed by CXCL8 and still by TNF. The HumanNet-XC reported HMGB-1, followed by GFAP and HGF as key interacting genes. Indeed, S100B and HMGB1 were both included in an extended frailty biomarker panel [110]. Moreover, considering the discussion on brain pathophysiological evidence linked to S100B as in the in-depth box, it is worth noting how VEGF and S100B are linked into the pathological evidence of major depressive disorders, both as potential predictors of treatment response [111].

The in-depth box number 3 points out the molecular cross-talk in cancer that involves muscle secretome. Using the gene related to the 18 myokines listed in Table 5 in addition with "Carcinoma" (MeSH Unique ID: D002277) GeneRecommender's DeepProphet 1 algorithm reported IL6 as the main gene of interest, followed by TNF and IFNγ; similarly, GeneRecommender's DeepProphet 2 algorithm pointed out IL6 and TNF as the first two recommendations, followed by IGFBP2 and VEGFA. These results highlight the primary role of inflammatory mediators such as IL6 and TNF in the molecular network of cancer initiation and progression [112].

The in-depth number 4 points out the importance of cytokines signaling in response to physical exercise. Using the genes related to the 9 interleukins as input, in addition to the tissue filtering for skeletal muscle, GeneRecommender's DeepProphet 1 algorithm reported GLI2, while GeneRecommender's DeepProphet 2 algorithm reported OSMR, as the main gene of interest. OSM was already reported in the first experts' list as related to chronic inflammation [113]; its receptor is highly expressed on several cells of nervous system [114]; therefore, the inflammatory acute and chronic response induced by physical exercise and mediated by the myokine OSM may play an important role in neuroprotection; indeed, this pathway has been already highlighted in high glucose conditions [115].

5. Limitations

Firstly, the use of four DS tools may have limited the possibility to obtain additional information by using alternative tools. However, those tools were chosen for their interesting features, and some of them included other tools as datasources.

The retrieved interactions used for suggesting putative myokines of interest include the interactions of myokines with their receptors, and the latter cannot be interpreted as putative myokines; this must be considered whne interpreting the recommendations. However, the inclusion of such interactions in the tools' algorithms cannot be currently avoided, and it is even useful because it allows to include any interactions of proteins with other molecules in the algorithms, thus providing more comprehensive outcomes.

Integrating healthy status with diseases such as diabetes and cancer may represent a bias in the selection of existent myokines and in the suggestions on the putative ones, as myokines in diseases may will play no role in healthy status. However, the current model did not focus on physiological vs pathophysiological myokines difference. Further models may provide ever updating references on myokines for health vs pathologies by recruiting specialized experts and setting DS methods as designed.

Another possible bias relies on the existing contradictory data for some myokines, thereby possibly biasing the selection; AI tools, as long as existing references report gene and protein interaction and co-citations and text mining support the presence of debated myokines, may produce biased outcomes. However, existing and developing features can allow users to filter data by including or not text-mining, selecting species, and focusing on tissues of interest. Therefore, the possibility of filtering by tissue-specific molecules, analytical procedures, exposure, and years of reference is going to refine the models.

Debate also exists on the role of EVs in muscle signaling: recent insights demonstrate, at least in animal models, that EVs released by skeletal muscle reach the circulation only to a subtle extent and rather accumulate within the muscle microenvironment [32,33]. Moreover, Vesiclepedia does not yet include human samples of skeletal muscle tissue in the database. However, the fact that existing or putative myokines have been found in EVs, although not directly from human skeletal muscle, suggest that those myokines may act, at least into muscle microenvironment, through EVs-related signaling.

6. Perspectives

For this study, the field of myokines was considered as a prototype for conducting integrated (experts and bioinformatic-assisted) recommendations for emerging insights. It is worth mentioning an online tool focused on skeletal muscle that integrates gene expression data of myofibers with biological pathways to create interaction networks to identify non-coding RNAs involved in muscle-specific functions [116]. Therefore, MyoData (https://myodata.bio.unipd.it/) was used with the 5 input genes recommended by all the three experts (bdnf, fgf21, igf1, fndc5, ostn), along with the first recommendations of GeneRecommender II (ins), HumanNet XC (fgf2), and GeneMANIA (ntf4). Results are shown in **Fig. 4** and highlight the role of miR322-5p, miR301a-3p, and miR27b-3p. Similar tools with several cells/tissues of interest can be really instrumental for scholars.

To complement **Supplementary table 1**, the AI recommendations for myokines and putative myokines have been detailed in **Table 4**. This table can serve as an additional basis for designing specific studies investigating the role of skeletal muscle secretome on several diseases.

Including both supervised and unsupervised learning methods, as well as encompassing algorithms focused on both protein interaction and gene, represent a comprehensive approach to take advantage of current DS tools for improving biomedical research. Several clustering methods are worth implementing to decipher systems of interest. Additional filters may account for selecting a timeframe and biological tissues of interest. The usefulness of text mining in AI-assisted reviewing procedures needs specific investigations. Further studies would compare in silico, in vitro and ex-vivo analyses to optimize the algorithms. Validation of predictive myokines may be conducted on existing single-

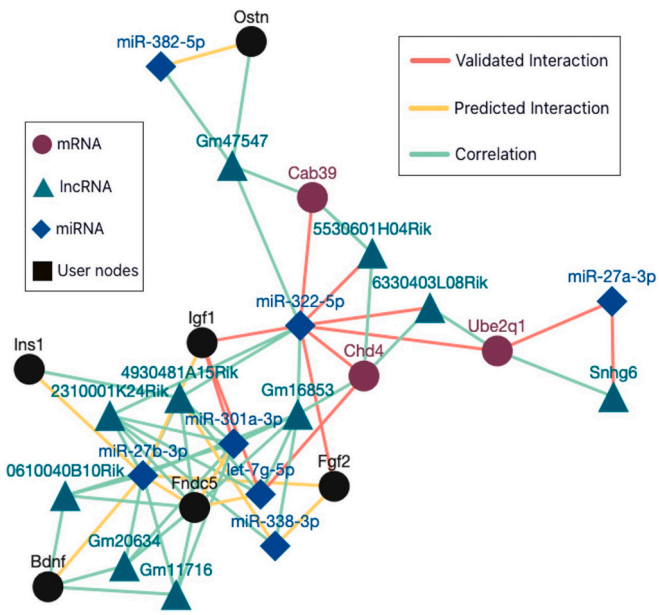

Fig. 4. A possible implementation of the workflow, using both experts' recommendations and top AI outcomes to model a molecular network of proteins and RNAs associations.

Table 4
List of myokines of interest recommended by AI tools and evaluated by experts

Myokine	Pathology/event	Effect	Reference
β-Klotho	Diabetes	Therapeutic	[170]
CCL2	DMD	Induced in muscle and blood, reduced in blood with age;	[171]
	Resistance training	variants are linked to strength	[172]
CCL3	ALS	Induced in CSF and blood	[173]
CCL20	Hypercholesterolemia	Induced in vascular smooth cells and plasma	[174]
CSF-3	ALS	Treatment;	[175]
	DMD	treatment;	[176]
	Muscle wasting	induced in blood	[177]
CXCL5	Aging	Induced in human blastocyst	[178]
	COPD	Induced in blood	[179]
	Obesity	Induced in blood	[180]
HSP10	Tumor, pregnancy	Induced in blood	[181]
IGF2	ALS	Protective for motor neurons	[182]
IGFBP3	Swimming training;	Altered in blood;	[183]
	Exercise during	Reduced in blood;	[97]
	hypoxia;	Induced in blood	[99]
	tennis playing		
IGFBP4	Aging	Increased in blood	[184]
IGFBP5	Aging;	Reduced in blood;	[184]
	Atherosclerosis, aging	Induced in endothelial tissue	[185]
IL1β	COPD;	Induced in airway epithelium;	[186]
	exercise	Induced in muscle	[187]
IL9	Dermatomyositis	Induced in blood and muscle	[188]
IL19	COPD	Induced in blood	[189]
IL20	Pancreatic cancer	Associated with poor survival	[190]
IL21	Myositis	Induced in blood and muscle	[191]
sIL6r	Exercise;	Induced in blood;	[192]
	COPD	Induced in sputum	[193]
TGFβ	Cachexia;	Induced in adipose tissue;	[194]
	Obesity	Induced in adipose tissue	[195]

cell references, accounting for fiber type heterogeneity [117], and on maps of dynamic response to muscle exercise [22]. A framework for designing further studies using in silico recommendations for optimizing molecular studies is finally depicted in Fig. 5. The inclusion of data on EVs released by human skeletal muscle within the continuous community annotation project of Vesiclepedia will allow to refine the knowledge of myokines-based signaling.

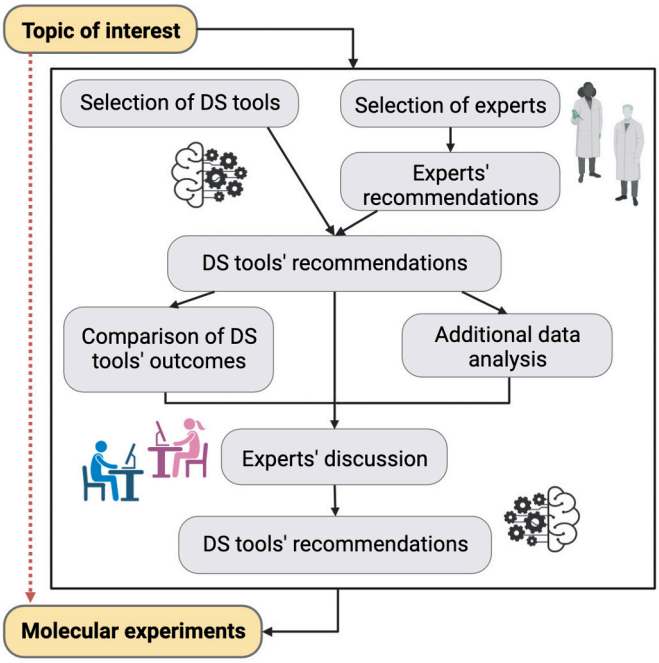

Fig. 5. Flow diagram of scholars&AI-assisted recommendations prior to molecular studies (black arrows and box), to be possible conducted rather than the common design (red arrow); image created in Biorender.com

7. Conclusions

The first suggestions and subsequent interpretations of results by a set of experts account for a proper definition of insights, to be furtherly assisted by DS predictions. All in all, bioinformatics and computational biology can be implemented into a network physiology framework to nurture myokine research. Computational analysis of muscle proteomics and molecular approaches to represent muscle cell-specific secretome and trafficking still continue to extend the world of myokines. Intriguing new approaches that rely on the isolation and profiling of extracellular fluid from muscle tissue may even enlarge the list of myokines [118]. This ever-growing list should be fine-tuned by differentiating in terms of health status and response to different exposures and interventions. To this extent, DS-assisted methods for reviewing existing evidence, recommending targets of interest, and predicting original scenarios are worth exploring enough that novel groundbreaking insights are likely to emerge from this paradigm.

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Availability of data

No datasets were generated or analyzed during the current study

Authors' contribution

Conceptualization, D.B.; Methodology, D.B., R.P., G.S., V.D.F.; Formal Analysis: D.B., M.B.; Investigation, M.E., A.N.V., N.A.V., S.C.P., and S.Y.W.; Writing – Original Draft, D.B., M.B., R.P., G.S., V.D.F., A.D. R.C., D.D.; Writing – Review & Editing, T.P., S.F.; Resources, D.B.; Visualization: D.B., M.B.; Supervision, T.P., S.F.

Declaration of Competing Interest

We, the authors, have no financial interests, no related patents, and no positions to declare and are not members of the journal's advisory board.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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