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A preliminary approach to creating an overview of lactoferrin multi-functionality utilizing a text mining method

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Abstract Lactoferrin is a multi-functional metal-binding glycoprotein that exhibits many biological functions of interest to many researchers from the fields of clinical medicine, dentistry, pharmacology, veterinary medicine, nutrition and milk science. To date, a number of academic reports concerning the biological activities of lactoferrin have been published and are easily accessible through public data repositories. However, as the literature is expanding daily, this presents challenges in understanding the larger picture of lactoferrin function and mechanisms. In order to overcome the "analysis paralysis" associated with lactoferrin information, we attempted to apply a text mining method to the accumulated lactoferrin literature. To this end, we used the information extraction system GENPAC (provided by Nalapro Technologies Inc., Tokyo). This information extraction system uses natural language processing and text mining technology. This system analyzes the sentences and titles from abstracts stored in the PubMed database, and can automatically extract binary relations that consist of interactions between genes/proteins, chemicals and diseases/functions. We expect that such information visualization analysis will be useful in determining novel relationships among a multitude of lactoferrin functions and mechanisms. We have demonstrated the utilization of this method to find pathways of lactoferrin participation in neovascularization, *Helicobacter pylori* attack on gastric mucosa, atopic dermatitis and lipid metabolism.

Keywords Lactoferrin · Text mining · Neovascularization · Angiogenesis · *Helicobacter pylori* · Atopic dermatitis · Lipid metabolism

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Introduction

Lactoferrin is a multi-functional protein that exhibits many biological activities as reported in the literature (Brock 2002). It is lactoferrin's multi-functionality that attracts a lot of researchers from the fields of clinical medicine, dentistry, pharmacology, veterinary medicine, nutrition and milk science. In a study of lactoferrin's structure–function relationship, three models were proposed to explain the multi-functionality of lactoferrin from a macroscopic view (Shimazaki 2004, 2005). The first model suggests that specific sites or fragments of the lactoferrin molecule constitute binding sites or active sites that enable lactoferrin function (Swiss army knife model). The second model involves lactoferrin binding and/or transferring various biological substances and behaving in a cooperative or competitive manner (carrier model). The last model suggests that

lactoferrin induces or suppresses the production of chemical mediators to control immune cells on intestinal epithelium, thereby affecting tissues or organs apart from the digestive tract (Billiard model).

To date, a number of academic reports concerning the biological activities of lactoferrin have been published and are easily accessible through public data repositories. However, as the literature is expanding daily, this presents challenges in understanding the larger picture of lactoferrin function and mechanisms. In order to overcome the information overload associated with lactoferrin, we applied a text mining method to the accumulated lactoferrin literature. To this end, we used the computer-assisted information extraction system GENPAC, which uses natural language processing and text mining technology (Kushida et al. 2009). We applied this method to certain functions and diseases to determine the possible participation of lactoferrin. Neovascularization or angiogenesis is relevant to cancer cells survival and growth (Carmeliet and Jain 2000; Norden et al. 2008). It is hypothesized that lactoferrin plays a role in preventing the formation of cancer cells. *Helicobacter pylori* is implicated in gastric cancer (Marshall 2003). Additionally, there are several publications reporting the efficacy of lactoferrin in attenuating *H. pylori*'s attack on gastric mucosa, such as the binding of recombinant human lactoferrin to *H. pylori* (Miehlke et al. 1996), and bovine lactoferrin decreasing the proportion of pepsinogen negative pyloric glands and the number of *H. pylori* and anti-*H. pylori* antibodies in an *H. pylori* infection model (Shimizu et al. 2000). Atopic dermatitis is caused by genetic, environmental and allergic factors. We attempted to determine the possibility of lactoferrin's participation in allergic processes. Lastly, we attempted to determine the relationship between lactoferrin and lipid metabolism. Through such trials, we expect to show that the information visualization analysis is valuable in determining novel relationships among a multitude of lactoferrin functions and their mechanisms.

Methods

Construction of lactoferrin's network of functional knowledge using text mining method

The flow of our method is shown in Fig. 1. Initially, we retrieved a list of articles pertaining to each keyword

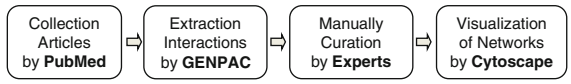

Fig. 1 Outline of the computer-assisted text mining method used for the lactoferrin interaction extraction process

from PubMed, a database that stores more than 19 million abstracts. Subsequently, MeSH terms corresponding to each lactoferrin function were used in keyword searches. Moreover, the "[majr]" tag was added to the MeSH term. Addition of the "[majr]" tag to the end of the keywords in PubMed searches results in the collection of articles in which the subject is a main topic. PubMed IDs were then retrieved from the list of the articles and were fed into GENPAC (for URL, see Table 1). GENPAC, which is developed and provided by NalaPro Technologies, Inc. (Tokyo), is an information extraction system for life science using natural language processing and text mining technology. This system contains a syntactic analyzer and three kinds of comprehensive lexicons that consist of genes/proteins, chemicals, and disease names. It can automatically extract binary relations, consisting of interactions between genes/proteins, chemicals and diseases, and interaction types among the sentences and titles of the retrieved citations. For example, GENPAC can extract an interaction relationship like "lactoferrin"—"inhibit"—"NF-kappaB" from the following sentence: "In these conditions, lactoferrin inhibited NF-kappaB activity by binding to—". Furthermore, it is expected that GENPAC will detect an interaction between biological entities A (lactoferrin) and C (disease a) from an interaction between biological entities A and B (function or related genes/proteins) and an interaction between biological entities B and C as shown in Fig. 2.

Comprehensive interaction information could be extracted by GENPAC, however, this information is likely to include false-positives. Therefore, we

Table 1 URL of database appeared in this article

Database	URL
Cytoscape	http://www.cytoscape.org/
Entrez gene	http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
GENPAC	http://www.nalapro.com/index_e.html
PubMed	http://www.ncbi.nlm.nih.gov/pubmed/
UniProt	http://www.uniprot.org/

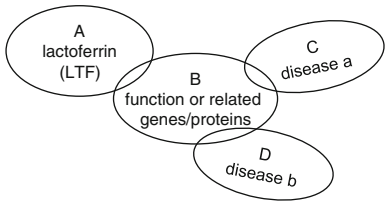

Fig. 2 Text mining provides the possibility of generating hypotheses concerning lactoferrin and its function or involvement in disease

examined the extraction errors of the interaction information obtained and all of the extraction errors were corrected or deleted manually. This combination of machine and manual curation is referred to as "hybrid curation" (Kushida et al. 2009).

Visualization of lactoferrin's function networks by Cytoscape

Lactoferrin function networks, interaction information concerning lactoferrin, were visualized using Cytoscape (for URL, see Table 1). Cytoscape is an open source bioinformatics software platform for visualizing molecular interaction networks and integrating these interactions with gene expression profiles and other state data (Shannon et al. 2003). The networks or maps prepared by Cytoscape are composed of three kinds of nodes (genes/proteins, chemicals, and diseases) and edges, which refer to the interaction types among nodes, such as "activate" and "bind". Each node includes an attribute of "displayName" which refers to the official NCBI symbol from the Entrez Gene database. Edges include certain attributes, such as "type of relationship" and "PubMed IDs".

Results and discussion

List of common genes/proteins between lactoferrin and each keyword

The left column of Table 2 contains the keywords used for the first step of Fig. 1, i.e., the collection of articles from PubMed. Using the citation list from PubMed for each keyword, GENPAC analysis gives us an output list composed of the gene/protein names, relations among them, PMID, year, journal and abstract (step 2 in Fig. 1). A variety of relations are utilized, including activation, stimulation, inhibition, suppression, induction, interaction, binding, up-regulation, down-regulation, etc. A list was prepared from this output data that contained each keyword, the number of references, genes/proteins and the number of genes/proteins in common with lactoferrin. Gene or protein IDs, identified by EntrezGene or Uniprot, are also recognized and extracted by GENPAC. As seen in Table 2, neovascularization, *H. pylori*, atopic dermatitis and lipid metabolism were selected for further analysis because the number of substances in common with lactoferrin was manageable.

Lactoferrin and the neovascularization pathway

The MeSH database defines "neovascularization" as "a pathologic process consisting of proliferation of blood vessels in abnormal tissues or in abnormal positions", and its entry term contains both neovascularization and angiogenesis. It is known that vascular endothelial growth factor (VEGF) binds to receptors and certain signaling pathways are activated which induce angiogenesis (Norrby 2004). To elucidate the participation of lactoferrin in these pathways, the following process for extracting the lactoferrin–neovascularization network information by GENPAC was performed. Initially, we collected article sets corresponding to lactoferrin and neovascularization or angiogenesis from PubMed. Next, we extracted lactoferrin/neovascularization interaction data from the article sets using GENPAC. We then checked and corrected the interaction data manually. Finally, the interaction data were visualized using Cytoscape, as shown in Fig. 3. In this figure, the nodes indicate proteins, genes and other substances, all of which are related to neovascularization or lactoferrin. The edges contain information pertaining to the literature or data denoting behaviors such as "activate/stimulate" or "suppress/inhibit" or "interact/bind". From this network, we identified pathways beginning with lactoferrin and proceeding to neovascularization related genes and substances, such as activation of kinase insert domain-containing receptor (KDR) expression by lactoferrin (Kim et al. 2006), the interaction between lactoferrin and estrogen receptor 1 (ESR1) (Stokes et al. 2004), and the interaction between ESR1 and KDR (Zaitseva et al. 2004). Finally, we created a model of lactoferrin's

Table 2 List of keywords and number of references, genes/proteins and common genes/proteins

Keywords (MeSH) biological functions	Number of		
	References	Substances	Common substances
Antioxidants	33781	251	45
Arteriosclerosis	67034	502	40
Candidiasis, cutaneous	749	0	0
Candidiasis, oral	2066	20	1
Cell aging	4469	100	15
Cell death	72017	1859	70
Cell proliferation	28969	1058	49
Dental caries	21744	18	3
Dermatitis, atopic	8567	49	23
Dry eye syndromes	8488	32	11
Halitosis	622	0	0
H. pylori	18807	179	26
Hepatitis C	32030	85	19
Immunization	43778	267	31
Inflammation	91740	780	54
Influenza, human	16519	31	6
Lipid metabolism[a]	23962	455	20
Neoplasm metastasis	26181	133	26
Neovascularization, physiologic	6072	141	29
Osteoporosis	23650	101	10
Peptic ulcer	52128	145	12
Periodontitis	12089	45	20
Reactive oxygen species	31545	709	47
Stomatitis	6218	34	9
Tinea Pedis	707	2	1
Xerostomia	7574	34	12
Lactoferrin	2354	79	–

Date of the data obtained at September 2009 ([a] January 2010)

possible participation in the neovascularization pathway (Fig. 4). This figure shows various several signal pathways in vascular endothelial cells started from VEGF receptor and ESR1 to induction of neovascularization.

Lactoferrin and *H. pylori* network

It is known that *H. pylori* attaches to the gastric mucosal epithelial cells and releases many kinds of substances such as CagA (a product of the cytotoxin-associated gene pathogenicity island), VacA (vacuolating toxin), lipopolysaccharide (LPS), urease, mucinase and other enzymes and chemicals (Marshall 2003; Merrell and Falkow 2004). The substances released from *H. pylori* induce physiological responses in the cells and finally leads to the development of ulcers. Urease produces ammonia as an enzymatic product that neutralizes gastric juice, and mucinase damages the gastric mucosa (Merrell and Falkow 2004). In general, the bacterial endotoxin LPS is recognized by Toll-like receptors (TLRs) and induces a robust pro-inflammatory response, and it is expected that lactoferrin binding of LPS would inhibit the inflammatory response (Elass et al. 2002). However, binding of *H. pylori* LPS by TLR5 exhibits a different reaction in gastric epithelial cells (Merrell and Falkow 2004). Moreover, VacA blocks T-cell modulation of the immune response (Gebert et al. 2003) and CagA induces actin reorganization

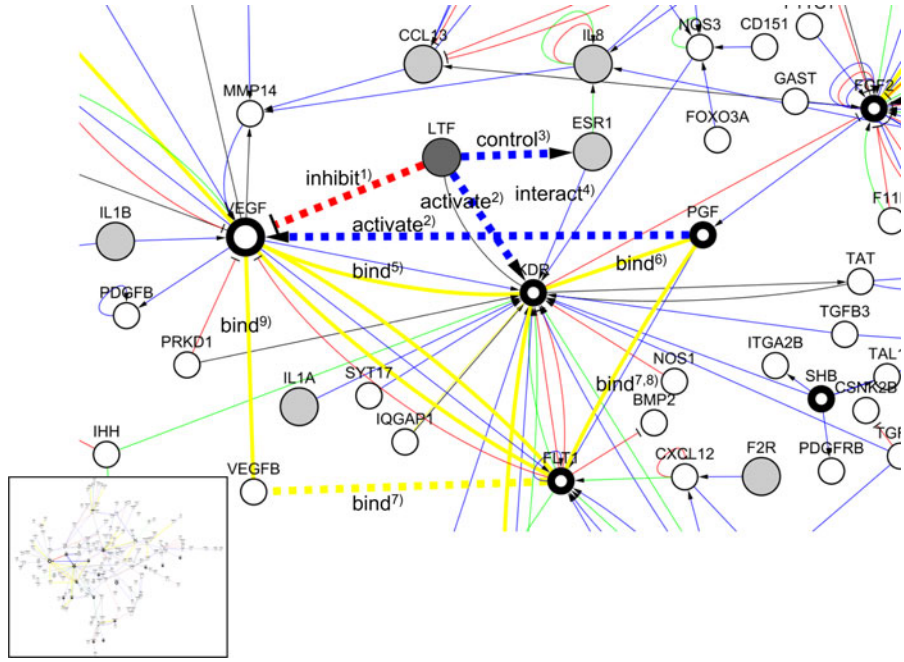

Fig. 3 A network of the interaction between lactoferrin and neovascularization prepared by Cytoscape. The overall view of the map is shown at the bottom left. *Small circles* or *nodes* represent genes or proteins extracted as neovascularization related entities. *Large nodes* represent substances related to both lactoferrin and neovascularization. *Bold nodes* represent neovascularization related substances defined by UniProt. *Arrow shape edges* indicate "activate" and *T figure shape edges* indicate "inhibit". *KDR* kinase insert domain-containing receptor, *LTF* lactoferrin, *PGF* placental growth factor, *PLT1* primed lymphocyte test-1, *VEGF* vascular endothelial growth factor. [1]Norrby (2004); [2]Kim et al. (2006); [3]Stokes et al. (2004); [4]Zaitseva et al. (2004); [5]Shibuya (2006a); [6]Zheng et al. (2007); [7]Shibuya (2006b); [8]Harrington et al. (2008); [9]Mould et al. (2005)

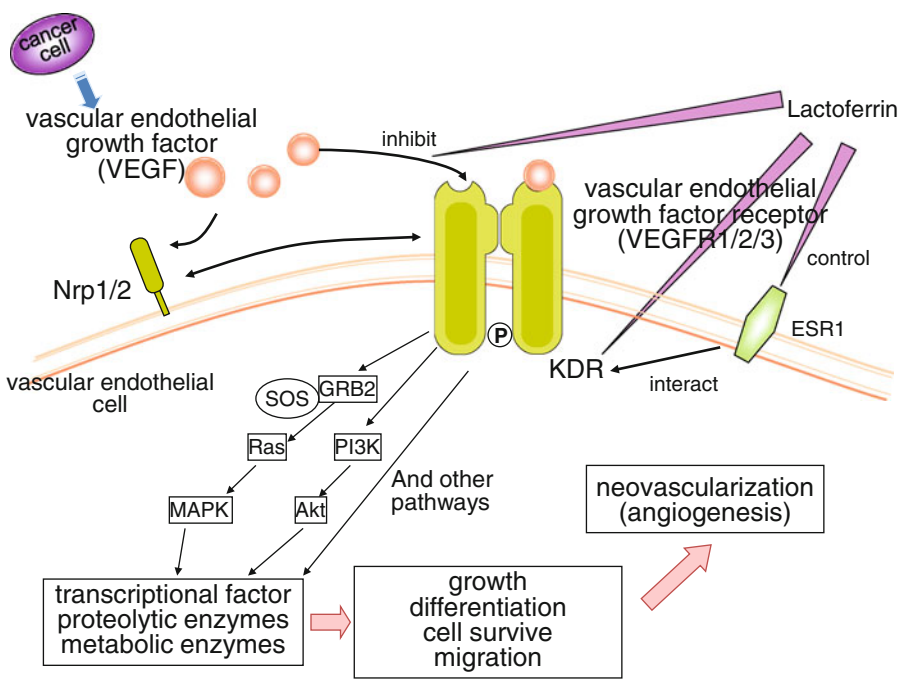

Fig. 4 Scheme of possible participation of lactoferrin in the neovascularization pathway. *Akt* Ser/Thr kinase, *ESR1* estrogen receptor 1, *GRB2* growth factor receptor-bound protein2, *MAPK* mitogen-activated protein kinase, *Nrp* neuropilin receptor, *PI3K* phosphatidylinositol-3-kinase, *Ras* rat sarcoma viral oncogene (small GTPase), *SOS* guanine nucleotide-exchange factor (GEF)

(Censini et al. 2001), inhibition of B-cell proliferation, suppression of B-cell apoptosis (Merrell and Falkow 2004) and interleukin-8 (IL8) induction of the gastric epithelium (Ernst and Gold 2000). It was reported that IL8 activates neutrophils to produce reactive oxygen species, which are injurious to the mucosal epithelium (Crabtree and Lindley 1994). Therefore, we focused on the relationship between lactoferrin and IL-8, as shown in Fig. 5. There are several pathways from lactoferrin to the IL8 node through the tumor necrosis factor (TNF) node (Lakshminarayanan et al. 1998). Similarly, there are many edges (evidence) indicating that TNF expression is controlled by lactoferrin (Crouch et al. 1992). There are other pathways from lactoferrin to IL8 through IL1β (Son et al. 2002; Mathy-Hartert et al. 2003). Figure 6 shows the possible participation of lactoferrin against *H. pylori*'s attack on gastric mucosal epithelial cells. From our analysis using GENPAC, it was suggested that the control of IL8 induction might be a possible pathway for the lactoferrin defense system.

The relationship between lactoferrin and atopic dermatitis

Figure 7 is a representation of the basic allergic pathways. IgE binds to mast cells, inducing the releases histamine, leukotriene and other cytokines involved in allergic inflammation (Turner and Kinet 1999; Galli et al. 2008). It is known that some food components effectively suppress atopic symptoms, including eicosapentaenoic acid (EPA)/docosahexaenoic acid (DHA) (Yu and Bjorksten 1998; Hwang et al. 2007) in fish oil, polyphenols (Akazome 2004), catechin (Noh et al. 2008) and flavonoids (Theoharides et al. 2004; Kawai et al. 2007) in plants. However, there is a dearth of literature supporting the effectiveness of lactoferrin on atopic dermatitis in vivo. Therefore, we attempted to compare the network of atopic dermatitis and flavonoid-related substances to lactoferrin by using the function of "merge networks" implemented in Cytoscape, as shown in Fig. 8. There are many interactions between flavonoids and allergy related substances, such as

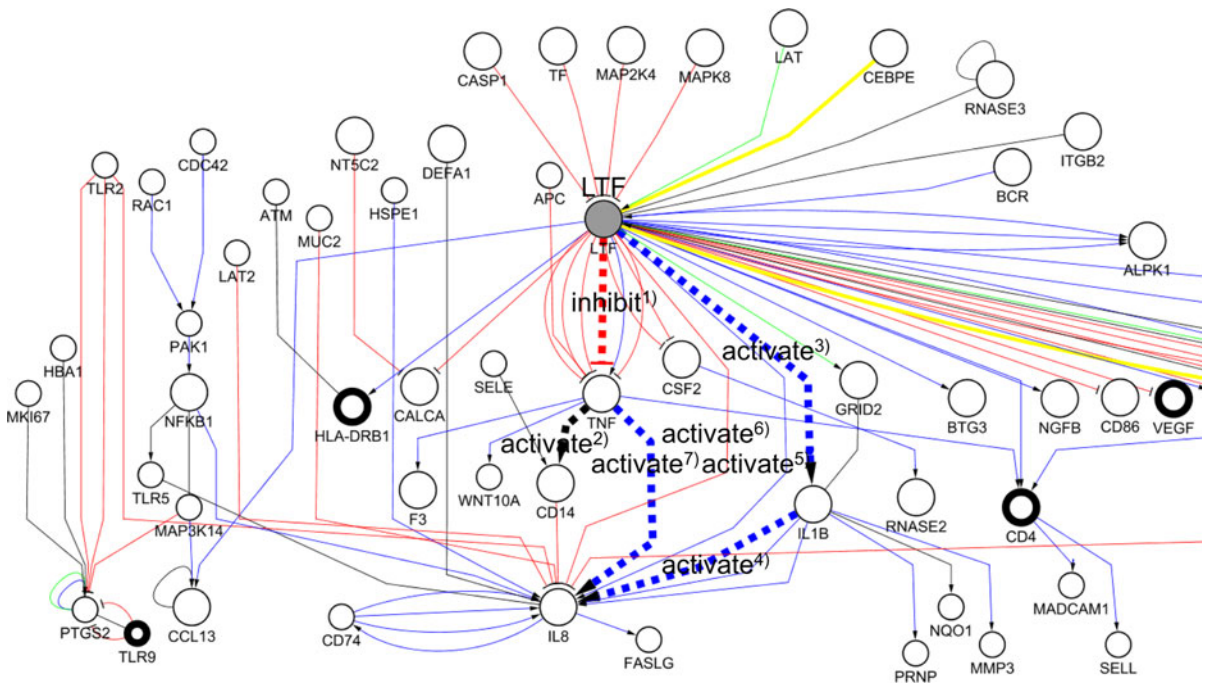

Fig. 5 Map of lactoferrin and *H. pylori* networks. *Large nodes* represent entities related to both lactoferrin and *H. pylori*. *Bold nodes* represent immune related substances by UniProt. *Arrow shape edges* indicate "activate" and *T figure shape edges* indicate "inhibit". *HLA-DRB1* major histocompatibility complex, class II, DR beta 1; *TLR9* toll-like receptor 9. [1]Crouch et al. (1992); [2]Lakshminarayanan et al. (1998); [3]Son et al. (2002); [4]Mathy-Hartert et al. (2003); [5]Komine et al. (2007); [6]Elass et al. (2002); [7]Ure et al. (1998)

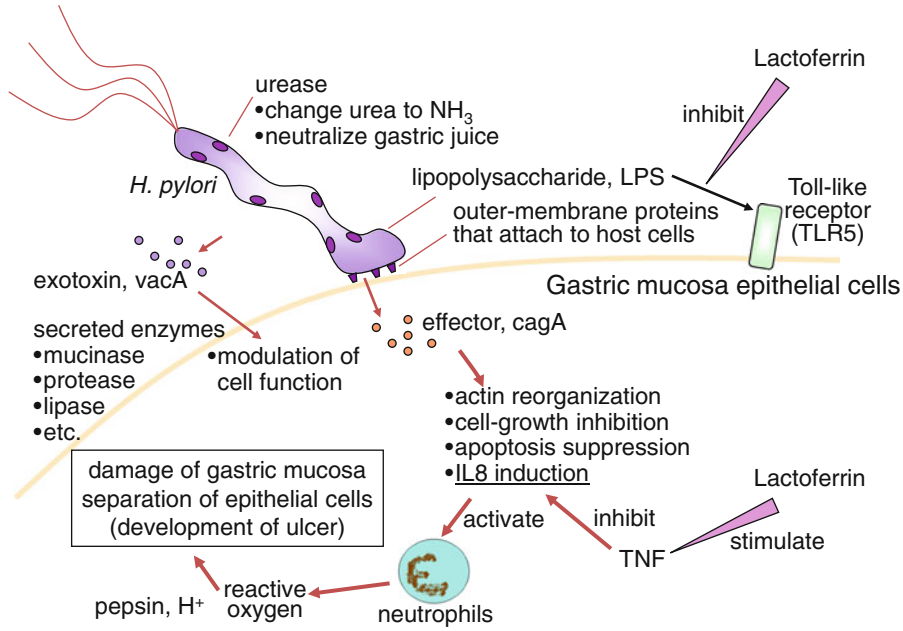

Fig. 6 Scheme of the possible role of lactoferrin in mitigating *H. pylori* attack on gastric mucosa

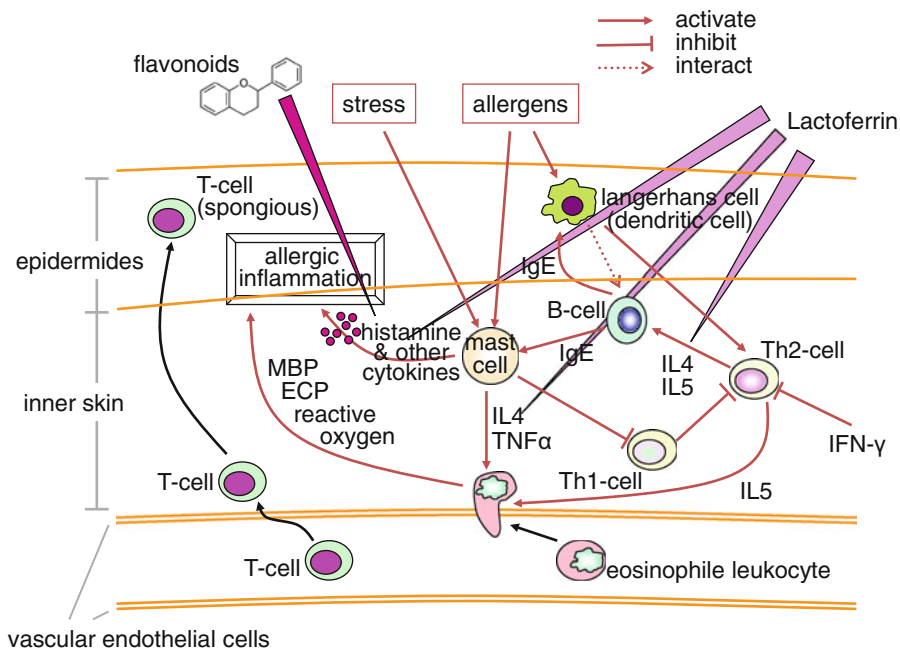

Fig. 7 Network of atopic dermatitis and related substances

histamine, interferon gamma (IFNG), intercellular adhesion molecule 1 (ICAM1), IL4 and other cytokines. Conversely, we found a lactoferrin pathway composed of four steps that acts through the suppression of cytokine production, control of cholesterol secretion, control of cyclic AMP production, and finally extended to the activation of IL4 production. Here, lactoferrin potentially decreases IL4 production and thereby suppresses cytokine production through a very indirect reaction.

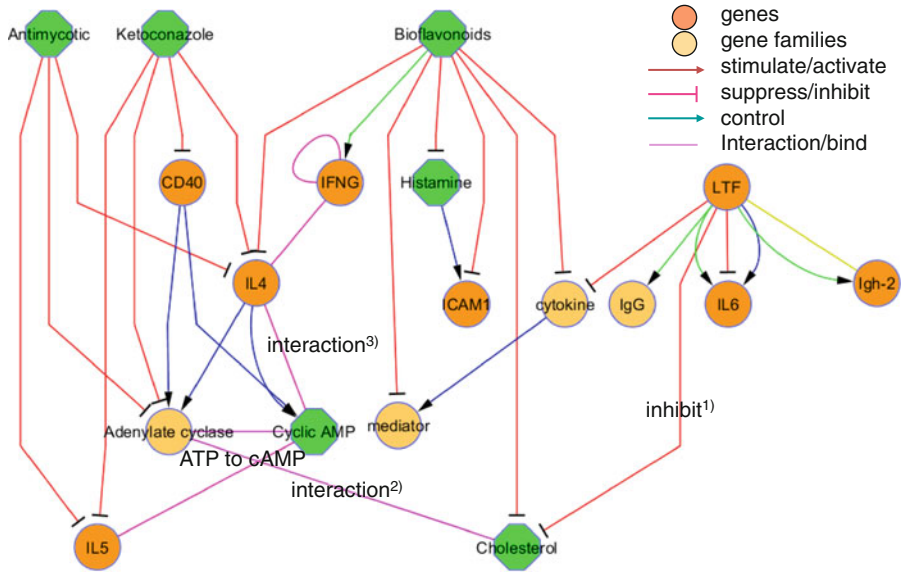

Fig. 8 Scheme of possible role of lactoferrin and flavonoids against atopic dermatitis [1]Takeuchi et al. (2004); [2]McMurchie et al. (1987); [3]Kanda et al. (2001)

Lactoferrin and lipid metabolism

Dietary fat absorbed in the small intestine is packaged into chylomicrons and transported to the liver through the blood stream. Triglycerides are hydrolyzed into fatty acids by lipoprotein lipase and they are taken up by the liver via the apolipoprotein E receptor (apoER). Dietary cholesterol is also packaged into high-density lipoprotein (HDL) particles and the conversion between HDL and very low-density lipoprotein (VLDL) occurs. Cholesterol ester from HDL is also transferred to VLDL remnant particles and is converted to LDL by the action of hepatic lipase. Finally, the liver and other tissues take up LDL by an endocytotic process that involves the LDL receptor. In these lipid metabolic pathways, points of contact with lactoferrin are not found. However, there are several reports suggesting a relationship between lipid metabolism and lactoferrin. The earliest work reports that lactoferrin inhibits the binding of modified LDL to macrophages (Kajikawa et al. 1994). Another study indicated that lactoferrin, orally administered to mice, showed a beneficial effect on plasma cholesterol levels and retarded hepatic lipid accumulation in mice fed a standard diet (Takeuchi et al. 2004). Moreover, an 8-week administration of enteric-coated lactoferrin significantly reduced visceral fat accumulation and the risk of metabolic syndrome with abdominal obesity (Morishita et al. 2009).

In response to experiments indicating that lactoferrin promotes lipid absorption as a consequence of the stimulation of bile acid synthesis with newborn rat fed lactoferrin (Shimono et al. 2008), we examined the relationship between lactoferrin and genes/proteins pertaining to cholesterol and bile acids. These results were obtained by DNA microarray analysis. Figure 9 shows the pathway from lactoferrin to TNF, IL1B and retinoblastoma 1 (RB1) to the nuclear receptor subfamily 1, group H, member 4 (HNF4A) and cholesterol 7α-hydroxylase (CYP7A). HNF4A is a nuclear transcription factor and may be essential for the development of the liver, kidney and intestine. On the other hand, CYP7A1 is an enzyme that catalyzes the conversion of cholesterol to bile acids. Figure 9 suggests the possibility of a new pathway for lactoferrin affecting lipid metabolism. Other pathways for explaining the participation of lactoferrin on lipid metabolism are currently being examined.

Possible detection of new lactoferrin pathways

Currently, many of the pathways regarding lactoferrin function and its involvement in diseases are not well understood. The information of networks, metabolic pathways, and signal transduction pathways is contained in a plethora of scientific articles, and biologists must manually extract this knowledge through reading

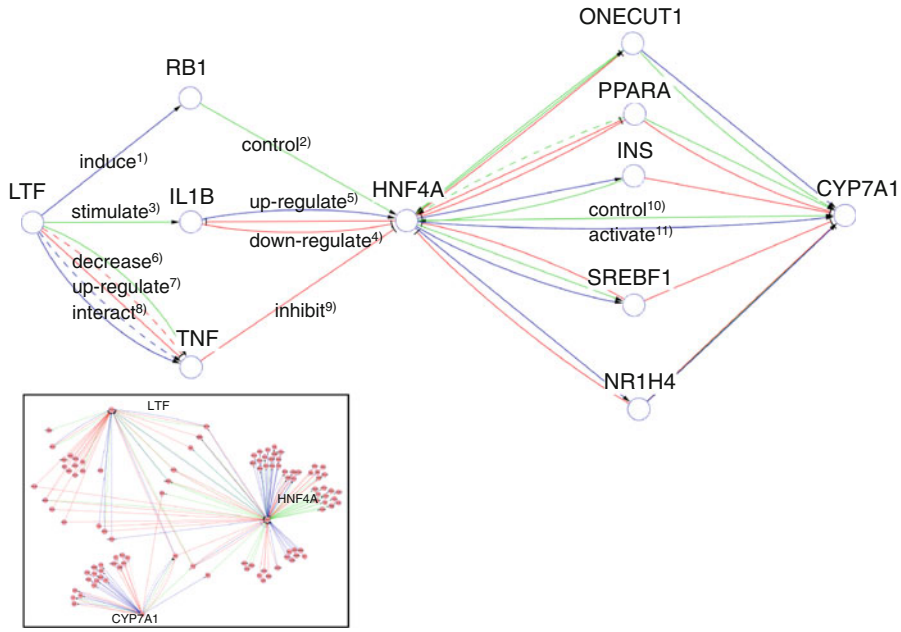

Fig. 9 Possible role of lactoferrin on lipid metabolism. The overall view of the map is shown at the bottom left. *CYP7A1* cholesterol 7α-hydroxylase, *HNF4A* hepatocyte nuclear factor 4, α, *IL1B* interleukin 1β, *INS* insulin, *NR1H4* nuclear receptor subfamily1, group H, member 4, *ONECUT1* one cut homeobox 1, *PPARA* peroxisome proliferator-activated receptor alpha, *RB1* retinoblastoma 1, *SREBF1* sterol regulatory element-1, *TNF* tumor necrosis factor. [1]Son et al. (2006); [2]Batsche et al. (2005); [3]Son et al. (2002); [4]Jahan and Chiang (2005); [5]Wang et al. (2001); [6]Zimecki et al. (2007); [7]Paul-Eugene et al. (1993), Adamik et al. (1998); [8]Choe and Lee (1999), Crouch et al. (1992); [9]De Fabiani et al. (2001); [10]Marrapodi and Chiang (2000); [11]Stroup and Chiang (2000)

articles. Therefore, the extraction of information about lactoferrin's functional networks is highly elaborate work. The computer-assisted text mining method described in this report can effectively extract lactoferrin information composed of genes, proteins, chemicals, functions and diseases from online databases. Herein, we attempted to discovery novel pathways among directly interacting entities, as well as linking biological phenomena to lactoferrin's signaling pathway map. Attempts to find new pathways have been proposed, such as Weeber's hypothesis generating model (Weeber et al. 2003) or Swanson's ABC model (Swanson 1986). In these reports, they indicated that laboratory experiments or clinical testing must be used to verify such hypotheses. This situation is the same as with the discovery of new pathways and biological networks regarding lactoferrin, as proposed in our report.

The benefits of hybrid curation method

We used the hybrid curation method for text mining in this study, which is the combination of machine curation by GENPAC and manual curation by the authors. It is clear that the time required for the current method is shorter than manual curation following actual reading of the articles. It is evident that this method supports high recall and precision for the network information, and that the network information can be efficiently collected by using the hybrid curation method. In general, the recall rate of information extracted by a machine curation is high, while information extracted by a manual curation is highly precise. According to estimates of the increase in number of PubMed articles, 2,000 or more articles per day are being published and this is leading to analysis paralysis for researchers. This has necessitated the development of new methods for the rapid and efficient collection of biological network and pathway knowledge. Therefore, our text mining method for finding new pathways might be a powerful assistant for the majority of researchers because it is useful for the explanation or analysis of existing experimental data as well as for planning new research.

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