Molecular Mechanism Study of Vitorgan Cell Molecular Therapy

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ABSTRACT

VitOrgan is a cell molecule drug extracted from bovine embryonic cells for anti-aging therapy since the 1960s in European countries. However, the molecular mechanisms involved remain unclear and underresearched. In this study, we applied human cytokine array with a group of 440 cytokine antibody, functional GO term enrichment and network technique to analyze the composition of vitOrgan(Mixed liquid: NeyPson Nr.5, NeyThel Nr.62, NeyNormin Nr.65, NeyDIL Nr.66, NeyDia Nr.67, NeyDIL Nr.70, NeyDesib Nr.78, NeyTroph Nr.96). The experimental results confirmed that the vitOrgan drug contains 123 cytokines, including ACE2, ADAM8, ADAM9, ADAMTS13, AFGF, ANGPT1, ANGPT2, ANG4, AR, AXIN, BAFF, BB84, TNFRSF17, BDNF, bFGF, BMP4, BMP5, BMPRIA, BMPRI B, BMP2, CDH4, CDH13, CADHERIN, CD40L, CD84, CD229, CTA4, CXCL9, CXCL10, CTPA, TNFRSF6B, DCN, ENA-78, FGF19, FGF21, FOLR1, FSH, GPRASP1, GPRASP2, GCP-2, GITRL, HCC4, ICAM3, IFNA1, IFNB1, IFNG, IGF1, IGF2, IGF3, IGFBP3, IGFBP4, IGFBP5, IL1A, IL2, IL4, IL10, IL13, IL17A, IL17F, IL21, IL27, IL36RN, IL1RI, IL1RAP, IL10RB, IL13RA2, IL17RA, IL17BR, IL17C, IL21R, IFN, JAM2, KLK14, LAG3, LDLR, LEP, LIF, CCL2, CCL8, CCL7, CL22, MER, MICA, MICB, CXCL9, CCL4, MMP-2, MMP-3, NACM1, MME, NOTCH1, NTFR4, PDCD1, PECAM-1, PGYPR1, TGFB3, TG, TEK, TACVR2, TLR4, TGFRA3, TWEAK, VEGF, VCAM1, WISP-1, EDA2R and ULBP2.

Cluster analysis through biomedical informatics techniques suggests cytokines of vitOrgan is a positive influence in cell biomedical regulation, including positive regulations of insulin-like growth factor receptor signaling pathway, cell proliferation, cell division, cell growth, ERK1 and ERK2 cascade, bone mineralization, epithelial cell proliferation, phosphatidylinositol 3-kinase signaling, protein kinase B signaling, glucose import, MAPK cascade, osteoblast differentiation pathway-restricted SMAD protein phosphorylation, cAMP metabolic process, tyrosine phosphorylation of Stat1 protein, participating immune response, proteoglycan biosynthetic process, inflammatory response, defense response to virus, chemokine-mediated signaling pathway, angiogenesis, growth factor activity, insulin secretion, DNA replication, lymphocyte chemotaxis, peptide catabolic process, endothelial cell apoptotic process and many other cell molecular activities. A preliminary interactome was built for the bovine vitOrgan proteins. Our results confirm the great potential of the vitOrgan as a clinical relevant therapeutic strategy.

Introduction

The treatment known as live cell therapy was firstly reported in Switzerland during the 1930s by Paul Niehans, which used organs, glands and fetuses of multiple species including sheep, cows and sharks [1,2]. In 1954, vitOrgan, freshly extracted from bovine, was founded by Karl E. Theurer in Germany, which has been used as a targeting cell molecular drug for 65 years [3-5]. Since 1965, vitOrgan has been widely used in many European countries and become the first popular brand of anti-aging and functional sports medicine in Europe, which was reported several times [6,7]. Moreover, antiallergic therapy with vitOrgan is currently an effective method for hypersensitivity of immune system demonstrated by F. Heiss of the University Medical Center Hamburg-Eppendorf (UKE) [8].
Materials and Methods

Materials

The concentrated injection solution, vitOrgan of NeyPsön Nr.5, NeyThel Nr.62, NeyNormin Nr.65, NeyDIL Nr.66, NeyDia Nr.67, were extracted from the bovine including Wagyu (black), Aberdeen Angus (black), Hereford (brown and white) and Brahman (grey and white).

Protein Array

We evaluated the quantitative concentrations of a total of 123 cytokines using the RayBio® G-Series Human Cytokine Antibody Array 440 (Cat# GSH-CAA-440, Array lot #Q0481618) according to the manufacturer’s instructions. Briefly, multiple cytokines specific capture antibodies were first bound to glass surfaces. After incubation with the plasma samples, the target cytokines were trapped on the solid surface as baits to capture the corresponding cytokines (or other mediators) in the applied vitOrgan samples (Mixed liquid: NeyPsön Nr.5, NeyThel Nr.62, NeyNormin Nr.65, NeyDIL Nr.66, NeyDia Nr.67, NeyDIL Nr.70, NeyDesib Nr.78, NeyTrophy Nr.96), and then incubated with a cocktail of pre-validated biotinylated secondary antibodies, and finally detected with Cy3-labeled streptavidin.

Gene Ontology and Pathway Analysis

Gene ontology (GO) and pathway analysis are suitable methods for integration genes with biological interaction and pathway networks to detect coordinated changes in functionally related genes. All the target genes are subjected to GO and pathway analysis in order to describe functional association of target genes. GO analysis was performed using GO pathway analysis using the open access software DAVID bioinformatics system and database (Database for Annotation, Visualization and Integrated Discovery, http://www.david.abcc.ncifcrf.gov website). Design and realization of software of cytokine statistical analysis for evaluation based on R language through KEGG (Kyoto Encyclopedia of Genes and Genomes https://www.genome.jp/kegg/) [16,17].

Data Analysis

Protein similarity analysis used the database of UniProt (https://www.uniprot.org/). A preliminary approach to the whole vitOrgan cytokines consisted of an elaboration for Network analysis with IPA software. As proteins in biological systems seldom work as independent entities, rather they are placed in widely interacting regulatory networks, the study of protein–protein interactions is a valid instrument to exploit in silico modelling to retrieve biologically relevant insights, through the individuation of pivotal webs of proteins and their potential main modulators. Network analysis software, such as IPA, determines and graphs unbiased networks, in which gene products are represented as nodes, and the biological relationship between two nodes is represented as an edge (line). All edges are supported by at least a reference from the literature, from a textbook, or from canonical information stored in the Ingenuity Pathways Knowledge Base for IPA [16,17]. The
databases and website including STRING (https://string-db.org/), National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) and GeneCardsSuite (https://www.genecards.org/).

**Statistical Analyses**

Data extraction can be done using the GAL file that is specific for this array along with the microarray analysis software (GenePix, ScanArray Express, ArrayVision, MicroVigene, etc.). Comparison of means between two groups was accomplished by the Student's t-test (two tailed). A P value <0.05 was considered statistically significant.

**Results**

**Cytokine Expression Profiles**

To identify the cytokines contained in vitOrgan injection solution for clinical applications, the cytokine profiles of vitOrgan samples (Mixed liquid: NeyPson Nr.5, NeyThel Nr.62, NeyNormin Nr.6, NeyDIL Nr.66, NeyDia Nr.67, NeyDIL Nr.70, NeyDesib Nr.78, NeyTrophi Nr.96) were analyzed using a RayBio® G-Series Human Cytokine Antibody Array 440. The top ten cytokines contained in vitOrgan were CD84 (911.74 pg/ml), LAG-3 (609.5 pg/ml), BMPR-IA (557.66 pg/ml), Thyroglobulin (533.43 pg/ml), IL-21 (450.47 pg/ml), IGFBP-4 (769.80 pg/ml), IL-17B R (350.96 pg/ml), IGFBP-3 (332.78 pg/ml), PDGF Rb (303.03 pg/ml) and WISP-1 (278.61 pg/ml) (Figure 1a & 1b), and CD84 was detected the highest level of the cytokine in samples (911.74 pg/ml) (Figure 1).

We firstly used Human Cytokine Antibody Array to detect the molecular composition of the concentrated vitOrgan injection extracted from the bovine and found that Vitorgan contained the following 123 cytokines (Table 1). Among these 123 cytokines, 30 cytokines had high concentrations (>100 pg/ml), including IL-13 R2, IL-21, IL-17B R, Procalcitonin, Thyroglobulin, ACE-2, FOLL1, GASP-2, LAG-3, Troponin I, VE-Cadherin, WISP-1, PDGF Rb, ANGPTL3, CTLLA4, ADAM12, BMPR-IB, Cadherin-4, Cadherin-13, CD84, DR3, Pref-1, BMPR-1A, BMPR-1I, RGM-B, BMP-5, IGFBP-5, IGFBP-3, IGFBP-4 and IGFBP-6 in the samples (Table 1). There were 79 cytokines at moderate concentrations (100 pg/ml-1 pg/ml), including Tie-2, AFP, IL-3, Leptin, MMP-2, MMP-3, 2B4, ADAM9, ANG-2, Dcr, FGF-19, IL-4, Kallikrein 14, Neprilysin, Notch-1, PD-1, ANG-4, BAFF, IL-27, BCMA, ICAM-3, IL-1 R4, IL-17R, IL-21R, L-Selectin, MICA, MICB, PECAM-1, VCAM-1, XEDAR, IL-17C, Mer, P-Cadherin, TF, TWEAK, ADAM8, CD229, Cystatin A, Desmoglein 2, FGF-19, JAM-B, SLAM, SP-D, Testican 2, TIM-3, TLR4, Axl, ENA-78, GCP-2, MCP-2, TECK, ADAMTS13, Angiotensinogen, B7-H1, GTR L, IL-1 R3, ULBP-2, AR, BDNF, BMP-4, NT-4, PIGF, SCF, SCF R and VEGF in the exposed samples (Table 1). Finally, fourteen cytokines have low concentrations (≤1 pg/ml), including IL-1 RI, IL-10 Rb, CA15-3, FSH, PGRPS, LDL-R, JAM-A, HCC-4, MCP-3, MDC, SDF-1a, TARC, TGFa and TGFb3 in the exposed samples (Table 1).

A complete list of identified proteins with international protein index accession number and the percentage of resemblance with human cytokines were summarized in Table 1. The array allowed us to conduct the expression profiling of hundreds of cytokines, chemokines, growth factors, soluble receptors and other proteins involved in the important signal pathways. Thus, our results showed that the vitOrgan injection solution contained a large number of molecules involved in the proteoglycan biosynthetic process, inflammatory response and defense responses. Furthermore, we searched the protein database of UniProt and found the most abundant molecules were 50%-90% resembling with the corresponding human protein sequences. In addition, 24 molecules did not show homology with human sequences, including IL-3, Leptin, PGRS, IL-27, L-Selectin, CTLLA4, IL-17C, TWEAK, ADAM12, Cystatin A, Pref-1, SLAM, TLR4, Axl, MCP-3, MDC, TECK, B7-H1, ULBP-2, BMP-4, BMP-5 and IGFBP-3 in the protein database of UniProt. We listed their highest homology with mouse, sheep and pig sequences (50%-90%) instead (Table 1).

| NO | Protein name | Concentration (pg/ml) | The UniProt Knowledgebase (UniProtKB) ID | Homology with human |
|----|--------------|-----------------------|------------------------------------------|---------------------|
| 1  | IL-13 R2     | 56.95                 | UniProtKB-A7B2C1 (A7B2C1_BOVIN)          | 50%                |
| 2  | Tie-2        | 17.4                  | UniProtKB-Q06807 (TIE2_BOVIN)            | 90%                |
| 3  | AFP          | 37.5                  | UniProtKB-Q35257 (FETA_BOVIN)            | 50%                |
| 4  | CA15-3       | 0.03                  | UniProtKB-Q8WML4 (MUC1_BOVIN)           |                     |
| 5  | FSH          | 0.43                  | UniProtKB-P04837 (FSHB_BOVIN)           | 50%                |
| 6  | IL-3         | 5.06                  | UniProtKB-P49875 (IL3_BOVIN)            | 50% (sheep)        |
| 7  | IL-21        | 450.40                | UniProtKB-Q76L05 (IL21_BOVIN)           | 50%                |
| 8  | Leptin       | 6.08                  | UniProtKB-P50595 (LEP_BOVIN)            | 50% (mouse)        |
| 9  | MMP-2        | 6.81                  | UniProtKB-Q9GE57 (MMP2_BOVIN)           | 90%                |
| 10 | MMP-3        | 1.45                  | UniProtKB-Q9XSF7 (Q9XSF7_BOVIN)          |                    |

Table 1: Proteins measured with cytokine antibody array.
| No. | Protein          | Score | GeneID                      | Percentage | Species       |
|-----|------------------|-------|-----------------------------|------------|---------------|
| 12  | Thyroglobulin    | 533.43| UniProtKB - P01267 (THYG_BOVIN) | 50%        |               |
| 13  | 2B4              | 34.1  | UniProtKB - D3K0R6 (AT2B4_BOVIN) | 50%        |               |
| 14  | ADAM9            | 1.01  | UniProtKB - F1MZJ5 (F1MZ5_BOVIN) | 90% (sheep)|               |
| 15  | ANG-2            | 20.41 | UniProtKB - O77802 (ANGP2_BOVIN) | 90%        |               |
| 16  | DcR3             | 45.97 |                            |            |               |
| 17  | FGF-19           | 46.28 | UniProtKB - E1BF8P8 (E1BF8P8_BOVIN) | 50%        |               |
| 18  | IGF-2            | 49.01 | UniProtKB - P07456 (IGF2_BOVIN) | 50%        |               |
| 19  | Kallikrein 14    | 2.14  | UniProtKB - F1MXS5 (F1MXS5_BOVIN) | 50%        |               |
| 20  | Neprilysin       | 9.78  |                            |            |               |
| 21  | Notch-1          | 25.65 | UniProtKB - F1MSM3 (F1MSM3_BOVIN) | 50% (mouse)|               |
| 22  | PD-1             | 9.73  | UniProtKB - A4FV85 (A4FV85_BOVIN) | 50%        |               |
| 23  | PGRP-S           | 0.7   | UniProtKB - Q8SPP7 (PGRP1_BOVIN) | 50% (mouse)|               |
| 24  | ACE-2            | 79.28 | UniProtKB - Q58DD0 (ACE2_BOVIN) | 50%        |               |
| 25  | ANG-4            | 9.5   | UniProtKB - Q24K15 (ANGP4_BOVIN) | 50%        |               |
| 26  | BAFF             | 8.21  | UniProtKB - B0LKN7 (B0LKN7_BOVIN) | 50%        |               |
| 27  | FOLR1            | 131.64| UniProtKB - E1BJL8 (E1BJL8_BOVIN) | 50%        |               |
| 28  | GASP-2           | 152.5 |                            |            |               |
| 29  | IL-17B R         | 350.96| UniProtKB - E1B7U9 (E1B7U9_BOVIN) | 50%        |               |
| 30  | IL-27            | 3.03  | UniProtKB - F6PU87 (F6PU87_BOVIN) | 50% (mouse)|               |
| 31  | LAG-3            | 609.5 | UniProtKB - E1B7C1 (E1B7C1_BOVIN) | 50%        |               |
| 32  | LDL R            | 0.87  | UniProtKB - P01131 (LDLR_BOVIN) | 50%        |               |
| 33  | Troponin I       | 89.92 | UniProtKB - P008057 (TNN13_BOVIN) | 90%        |               |
| 34  | VE-Cadherin      | 94.74 | UniProtKB - Q6URK6 (CADH5_BOVIN) | 50%        |               |
| 35  | WISP-1           | 278.61| UniProtKB - F1MS54 (F1MS54_BOVIN) | 50%        |               |
| 36  | BCMA             | 13.99 | UniProtKB - M5F667 (M5F667_BOVIN) | 50%        |               |
| 37  | ICAM-3           | 31.2  | UniProtKB - Q28125 (ICAM3_BOVIN) | 50%        |               |
| 38  | IL-1R4           | 2.27  |                            |            |               |
| 39  | IL-1 RI          | 0.01  | UniProtKB - Q2LGB5 (TOLIP_BOVIN) | 50%        |               |
| 40  | IL-10 Rb         | 0.81  | UniProtKB - Q08DU4 (Q08DU4_BOVIN) | 50%        |               |
| 41  | IL-17R           | 7.59  | UniProtKB - F1N2C5 (F1N2C5_BOVIN) | 50%        |               |
| 42  | IL-21R           | 40.6  | UniProtKB - E1B8E5 (E1B8E5_BOVIN) | 50%        |               |
| 43  | L-Selectin       | 5.77  | UniProtKB - P98131 (LAM1_BOVIN) | 90% (sheep)|               |
| 44  | MICA             | 9.75  | UniProtKB - F1MH07 (MICA1_BOVIN) | 50%        |               |
| 45  | MICB             | 2.158 |                            |            |               |
| 46  | PDGF Rb          | 303.03|                            |            |               |
| 47  | PECAM-1          | 4.64  | UniProtKB - P51B66 (PECA1_BOVIN) | 50%        |               |
| 48  | VCAM-1           | 23.03 | UniProtKB - A7MBB0 (A7MBB0_BOVIN) | 50%        |               |
| 49  | XEDAR            | 41    |                            |            |               |
| 50  | ANGPTL3          | 142.59| UniProtKB - Q2KJB3 (Q2KJB3.BOVIN) | 50%        |               |
|  | Protein | UniProtKB | Description |
|---|---|---|---|
| 51 | CTLA4 | Q28090 (Q28090_BOVIN) | 90% |
| 52 | IGFBP-5 | Q05717 (IBP5_BOVIN) | 90% (sheep) |
| 53 | IL-17C | G3MYZ5 (G3MYZ5_BOVIN) | 90% |
| 54 | Mer | F1N381 (F1N381_BOVIN) | 50% (sheep) |
| 55 | P-Cadherin | P19535 (CADH3_BOVIN) | 50% |
| 56 | TF | P10931 (TF_BOVIN) | 50% |
| 57 | TWEAK | F1AGC7 (F1AGC7_BOVIN) | 90% (sheep) |
| 58 | ADAM8 | F1MIY8 (F1MIY8_BOVIN) | 50% |
| 59 | ADAM12 | F1MWS2 (F1MWS2_BOVIN) | 90% (sheep) |
| 60 | BMPR-IB | Q0Q7Q1 (Q0Q7Q1_BOVIN) | 90% |
| 61 | Cadherin-4 | F1MW29 (F1MW29_BOVIN) | 90% |
| 62 | Cadherin-13 | Q3B7N0 (CAD13_BOVIN) | 90% |
| 63 | CD84 | F1N7U9 (F1N7U9_BOVIN) | 50% |
| 64 | CD29 | Q3ZBB1 (SH21A_BOVIN) | 90% |
| 65 | Cystatin A | P01035 (CYTC_BOVIN) | 90% (sheep) |
| 66 | Desmoglein 2 | F1MFC2 (F1MFC2_BOVIN) | 50% |
| 67 | DR3 | E1BDA6 (E1BDA6_BOVIN) | 50% |
| 68 | FGF-21 | Q9XT56 (JAM1_BOVIN) | 50% |
| 69 | JAM-A | Q9XT56 (JAM1_BOVIN) | 50% |
| 70 | JAM-B | 0.19 | 50% |
| 71 | Pref-1 | Q46370 (Q46370_BOVIN) | 90% (sheep) |
| 72 | SLAM | Q1RML5 (Q1RML5_BOVIN) | 90% (sheep) |
| 73 | SP-D | 23.16 | 90% (sheep) |
| 74 | Testican 2 | 41.12 | 90% |
| 75 | TIM-3 | 21.91 | 90% |
| 76 | TLR4 | Q6WCD5 (Q6WCD5_BOVIN) | 50% (mouse) |
| 77 | Axin | F1N0D3 (F1N0D3_BOVIN) | 50% (mouse) |
| 78 | ENA-78 | 16.25 | 90% (sheep) |
| 79 | GCP-2 | CXCL6_BOVIN | 50% |
| 80 | HCC-4 | 0.94 | 90% (sheep) |
| 81 | MCP-2 | Q91411 (CCL8_BOVIN) | 50% |
| 82 | MCP-3 | Q9GLX0 (ACKR1_BOVIN) | 90% (sheep) |
| 83 | MDC | E1BI26 (E1BI26_BOVIN) | 50% (sheep) |
| 84 | SDF-1a | P25930 (CXCR4_BOVIN) | 90% |
| 85 | TARC | F1MIF0 (F1MIF0_BOVIN) | 50% |
| 86 | TECK | Q1RMQ0 (Q1RMQ0_BOVIN) | 50% (pig) |
GO Annotation Analysis of vitOrgan Cytokines

Network analysis through DAVID yielded the identification of 91 networks, as reported in Table 2. Most of these networks are accounted for biological functions related to extracellular space (22 cytokines), extracellular region (11 cytokines) and positive regulation of cell proliferation (8 cytokines). Networks involved in immune response were present as well (7 cytokines). The rest of the networks usually involve 2-5 cytokines from vitOrgan. Most of the networks were intertwined, as they shared multiple entries. Indeed, certain categories of proteins were often recurring in multiple networks, because of their multifaceted roles in cell activities and regulations, such as IGFBP3 (network 1, 10, 17, 49, 68, 77, 78 and 80), chemokines (network 1, 2, 3, 11, 13, 22, 26, 29, 31, 36, 41, 50, 59, 79 and 87) and growth factors (network 1, 2, 11, 12, 14, 16 and 23). The most significantly enriched pathways with the P-value are shown in Table 2.

Three out of the ten most abundant cytokines shown on Figure 1 participate in different signaling pathways in the network analysis. For example, BMPR1A involved in 14 signal pathways using network analysis, including external side of plasma membrane, caveola, immune response, positive regulation of bone mineralization, positive regulation of epithelial cell proliferation, positive regulation of osteoblast differentiation, positive regulation of pathway-restricted SMAD protein phosphoryl, embryonic organ development, positive regulation of mesenchymal cell proliferation, mesoderm formation, chondrocyte differentiation, glycoprotein binding, receptor signaling protein serine/threonine kinase activity, BMP receptor activity (Table 2). IGFBP3 involved in 8 signal pathways by network analysis, including extracellular space, positive regulation of insulin-like growth factor receptor signal, regulation of cell growth, positive regulation of myoblast differentiation, response to insulin, insulin-like growth factor binding, insulin-like growth factor I binding, insulin-like growth factor II binding, fibronectin binding (Table 2). IGFBP4 involved in 7 signal pathways by network analysis, including extracellular space, positive regulation of insulin-like growth factor receptor signal, regulation of cell growth, positive regulation of MAPK cascade, positive regulation of insulin-like growth factor receptor signal, regulation of cell growth, positive regulation of MAPK cascade, positive regulation of insulin-like growth factor receptor signal, regulation of cell growth, positive regulation of MAPK cascade, positive regulation of insulin-like growth factor II binding, IGFBP-3 and IGFBP-
participated signaling pathways together, including extracellular space, positive regulation of insulin-like growth factor receptor signal, regulation of cell growth, insulin-like growth factor I binding, insulin-like growth factor II binding (Table 2).

**Table 2:** Identified networks in vit Organ cytokines.

| NO | Term                                                                 | Count | Genes                                                                                                           | PValue      |
|----|----------------------------------------------------------------------|-------|-----------------------------------------------------------------------------------------------------------------|-------------|
| 1  | Extracellular space                                                  | 22    | TG, ADAMTS13, IGFBP6, BMPR2, CXCL9, TGFB3, IGF1, IGF2, DCN, CCL4, GAS6, CXCL10, LEP, ACE, IFNA1, IFNB1, IFNG, ACE2, TGFA, IGFBP3, IGFBP4, IGFBP5 | 1.22E-15    |
| 2  | Extracellular region                                                 | 11    | LEP, FGFR2, LGF, VWF, BDNF, IGFBP6, IGF2, FGFR2, FGFR1, CCL4, GAS6, CXCL10                                    | 4.29E-07    |
| 3  | External side of plasma membrane                                     | 6     | ACE, IFNG, CXCL9, CTLA4, BMPR1A, CXCL10                                                                       | 3.76E-05    |
| 4  | Cell surface                                                         | 5     | TEK, BMPR2, ACE2, ITG8, ADAM8                                                                                 | 0.008398483 |
| 5  | Proteinaceous extracellular matrix                                   | 4     | VWF, SPOCK2, ADAMTS13, DCN                                                                                     | 0.010401848 |
| 6  | Extracellular matrix                                                 | 3     | VWF, TGFB3, DCN                                                                                               | 0.03341784  |
| 7  | Integral component of plasma membrane                               | 6     | TEK, ICAM3, BMPR2, TGFA, TLR4, ADA8                                                                           | 0.067253061 |
| 8  | Membrane                                                             | 6     | ACE, ICAM3, BMPR2, CTLA4, ITG8, BMPR1B                                                                        | 0.073056777 |
| 9  | Caveola                                                              | 2     | BMPR2, BMPR1A                                                                                                  | 0.097443665 |
| 10 | Positive regulation of insulin-like growth factor receptor signal    | 4     | IGFBP6, IGFBP3, IGFBP4, IGFBP5                                                                                | 2.07E-06    |
| 11 | Positive regulation of cell proliferation                            | 8     | FGFR2, FGFR1, LGF, IGF1, TGFA, FGFR2, BMPR1B, CXCL10                                                          | 2.87E-06    |
| 12 | Positive regulation of ERK1 and ERK2 cascade                         | 6     | FGFR2, FGFR1, TEK, FGFR2, CCL4, GAS6                                                                         | 2.74E-05    |
| 13 | Immune response                                                      | 7     | IGF, CXCL9, CTLA4, TNFAIP3, CCL4, BMPR1A, CXCL10                                                              | 5.23E-05    |
| 14 | Positive regulation of cell division                                | 4     | FGFR2, TGFB3, TGFA, IGF2                                                                                       | 7.19E-05    |
| 15 | Positive regulation of bone mineralization                           | 4     | BMPR2, TGFB3, BMPR1B, BMPR1A                                                                                  | 7.19E-05    |
| 16 | Positive regulation of epithelial cell proliferation                 | 4     | FGFR2, IGF1, TGFA, BMPR1A                                                                                      | 1.28E-04    |
| 17 | Regulation of cell growth                                           | 4     | IGFBP6, IGFBP3, IGFBP4, IGFBP5                                                                                | 1.42E-04    |
| 18 | Positive regulation of osteoblast differentiation                    | 4     | BMPR2, IGF1, BMPR1B, BMPR1A                                                                                     | 1.90E-04    |
| 19 | Proteoglycan biosynthetic process                                   | 3     | BMPR2, IGF1, BMPR1B                                                                                            | 2.38E-04    |
| 20 | Positive regulation of phosphatidylinositol 3-kinase signaling       | 4     | LEP, TEK, IGF1, DCN                                                                                             | 3.13E-04    |
| 21 | Positive regulation of protein kinase B signaling                    | 4     | LEP, TEK, ADAM8, GAS6                                                                                           | 3.64E-04    |
| 22 | Defense response to virus                                           | 5     | IFNA1, IFNB1, IFNG, CXCL9, CXCL10                                                                             | 6.43E-04    |
| 23 | Positive regulation of glucose import                               | 3     | FGFR1, IGF1, FGFR2                                                                                              | 0.00106834  |
| 24 | Lung alveolus development                                           | 3     | FGFR2, LGF, BMPR2                                                                                              | 0.001694871 |
| 25 | Positive regulation of transcription from RNA polymerase II promo    | 7     | FGFR2, LGF, IGF1, BMPR2, IGF1, DCN, BMPR1B                                                                   | 0.003423715 |
| 26 | Inflammatory response                                                | 5     | CXCL9, TLR4, BMPR1B, CCL4, CXCL10                                                                             | 0.003719935 |
| 27 | Angiogenesis                                                         | 4     | FGFR2, LEP, TGFA, ADAM8                                                                                         | 0.00403412  |
| 28 | Positive regulation of tumor necrosis factor (ligand) superfamily    | 2     | IFNG, ADAM8                                                                                                    | 0.006849896 |
| 29 | Chemokine-mediated signaling pathway                                 | 3     | CXCL9, CCL4, CXCL10                                                                                            | 0.007080854 |
| 30 | Positive regulation of MAPK cascade                                  | 3     | LEP, LGF, IGFBP4                                                                                               | 0.007080854 |
| 31 | Positive regulation of cell proliferation                            | 4     | FGFR2, CXCL9, TGFB3, CXCL10                                                                                     | 0.007769512 |
| 32 | Positive regulation of pathway-restricted SMAD protein phosphoryl    | 3     | BMPR2, TGFB3, BMPR1A                                                                                            | 0.007844344 |
| No. | Process/Function                                      | Biological Processes | Pathways/Components            | p-Value |
|-----|------------------------------------------------------|----------------------|--------------------------------|---------|
| 33  | Neutrophil chemotaxis                               |                      | IFNG, ITGB2, CCL4              | 0.008239423 |
| 34  | Negative regulation of chondrocyte proliferation    |                      | BMPR2, BMPR1B                  | 0.010257679 |
| 35  | Endochondral bone morphogenesis                     |                      | BMPR2, BMPR1B                  | 0.010257679 |
| 36  | Positive regulation of cAMP metabolic process       |                      | CXCL9, CXCL10                  | 0.013654067 |
| 37  | Receptor-mediated virion attachment to host cell    |                      | ACE2, GAS6                     | 0.013654067 |
| 38  | Lung lobe morphogenesis                             |                      | FGFR2, BMPR1A                  | 0.017039098 |
| 39  | Positive regulation of insulin-like growth factor receptor signal | | IGF1, IGFBP4                  | 0.023775236 |
| 40  | Negative regulation of gene expression              |                      | BG19, ACE, IFNG                | 0.030466388 |
| 41  | Positive regulation of cAMP-mediated signaling      |                      | CXCL9, CXCL10                  | 0.030466388 |
| 42  | Embryonic organ development                          |                      | BMPR2, BMPR1A                  | 0.033795185 |
| 43  | Transmembrane receptor protein serine/threonine kinase signaling | | BMPR2, BMPR1B                  | 0.033795185 |
| 44  | Positive regulation of membrane protein ectodomain proteolysis | | IFNG, ADAM8                   | 0.033795185 |
| 45  | Positive regulation of cartilage development        |                      | BMPR2, BMPR1B                  | 0.033795185 |
| 46  | Positive regulation of tyrosine phosphorylation of Stat1 protein | | IGF1, IFNG                    | 0.033795185 |
| 47  | Cell-substrate adhesion                              |                      | VWF, GAS6                      | 0.033795185 |
| 48  | Positive regulation of mitotic nuclear division     |                      | IGF1, TGFA                     | 0.033795185 |
| 49  | Positive regulation of myoblast differentiation      |                      | CXCL9, IGFBP3                  | 0.04019405 |
| 50  | Positive regulation of leukocyte chemotaxis         |                      | CXCL9, CXCL10                  | 0.043714901 |
| 51  | chondrocyte development                              |                      | BMPR2, BMPR1B                  | 0.043714901 |
| 52  | Positive regulation of protein secretion            |                      | TGF83, IGF1                    | 0.050272841 |
| 53  | Positive regulation of mesenchymal cell proliferation |                      | FGFR2, BMPR1A                  | 0.050272841 |
| 54  | Negative regulation of smooth muscle cell proliferation |                      | IFNG, TNFAIP3                 | 0.050272841 |
| 55  | Regulation of insulin secretion                      |                      | LEP, IFNG                      | 0.053535359 |
| 56  | Positive regulation of DNA replication              |                      | TGF83, IGF1                    | 0.053535359 |
| 57  | Positive regulation of phagocytosis                 |                      | ITGB2, GAS6                    | 0.056786955 |
| 58  | Negative regulation of endothelial cell apoptotic process |                      | TNFAIP3, GAS6                 | 0.056786955 |
| 59  | Positive regulation of release of sequestered calcium ion into cytoplasm | | CXCL9, CXCL10                  | 0.056786955 |
| 60  | Regulation of multicellular organism growth          |                      | FGFR2, IGF1                    | 0.060027666 |
| 61  | Peptide catabolic process                            |                      | ACE, ADAMTS13                  | 0.060027666 |
| 62  | Lymphocyte chemotaxis                               |                      | ADAM8, CCL4                    | 0.063257527 |
| 63  | Phagocytosis                                         |                      | LEP, ITGB2                     | 0.063257527 |
| 64  | Negative regulation of tumor necrosis factor production |                      | TNFAIP3, GAS6                 | 0.066476573 |
| 65  | Positive regulation of endothelial cell migration   |                      | TEK, BMPR2                     | 0.06968484 |
| 66  | Positive regulation of tumor necrosis factor production |                      | LEP, IFNG                     | 0.072882363 |
| ID | Process                                      | Gene(s)                          | p-value          |
|----|---------------------------------------------|----------------------------------|------------------|
| 67 | Mesoderm formation                          | BMPR2, BMPR1A                    | 0.072882363      |
| 68 | Response to insulin                         | LEP, IGFBP3                      | 0.072882363      |
| 69 | Blood vessel remodeling                     | LIF, BMPR2                       | 0.076069176      |
| 70 | Protein kinase B signaling                  | IGF1, GAS6                       | 0.079245316      |
| 71 | Glucose metabolic process                   | LEP, IGF1                        | 0.085565711      |
| 72 | Fibroblast growth factor receptor signaling pathway | FGFR2, FGFR19 | 0.085565711      |
| 73 | Chondrocyte differentiation                 | BMPR1B, BMPR1A                   | 0.098079936      |
| 74 | Positive regulation of fibroblast proliferation | IGF1, GAS6                      | 0.098079936      |
| 75 | Glycoprotein binding                        | VWF, ACE2, TGFA, ITGB2, BMPR1B, BMPR1A | 9.60E-08   |
| 76 | Growth factor activity                      | LEP, BDNF, TGFB3, IGF1, TGFA, IGF2 | 6.04E-07   |
| 77 | Insulin-like growth factor I binding        | IGFBP6, IGFBP3, IGFBP4, IGFBP5  | 6.87E-07         |
| 78 | Insulin-like growth factor II binding       | IGFBP6, IGFBP3, IGFBP4, IGFBP5  | 6.87E-07         |
| 79 | Chemokine activity                          | CXCL9, CCL4, CXCL10              |                 |
| 80 | Peptidyl-dipeptidase activity               | ACE, ACE2                        | 0.010014361      |
| 81 | Fibronectin binding                         | IGFBP3, IGFBP5                   | 0.010014361      |
| 82 | Receptor signaling protein serine/threonine kinase activity | BMPR2, BMPR1B, BMPR1A | 0.012084755 |
| 83 | Hormone activity                            | LEP, IGF1, IGF2                  | 0.012551697      |
| 84 | BMP receptor activity                        | BMPR2, BMPR1A                    | 0.013330745      |
| 85 | Transmembrane receptor protein serine/threonine kinase activity | BMPR2, BMPR1B | 0.023215174 |
| 86 | Collagen binding                            | VWF, DCN                         | 0.036244659      |
| 87 | CXC chemokine receptor binding              | CXCL9, CXCL10                    | 0.04590564       |
| 88 | Cytokine activity                           | IFNA1, IFNG, TGFB3               | 0.059119952      |
| 89 | Integrin binding                            | VWF, ICAM3                       | 0.061798266      |
| 90 | Endopeptidase activity                      | ACE, ACE2                        | 0.068082934      |
| 91 | Protein tyrosine kinase activity            | TEK, AXL                         | 0.071209889      |

**Figure 1:** Cytokine antibody array of vitOrgan

A. Representative array images of top ten cytokine in vitOrgan. The 10 marker panel was annotated above and under the images.

B. Relative signal intensity of top ten cytokine contained in vitOrgan.
Statistical analysis was conducted on the enrichment of target genes that belong to each GO term and statistically significant values were calculated for each category. The significantly enriched GO terms were related to extracellular space (GO:0005615), external side of plasma membrane (GO:0000897), extracellular region (GO:0005576), cell surface (GO:0009986), proteinaceous extracellular matrix (GO:0005578), integral component of plasma membrane (GO:0005887), caveola (GO:0005901), immune response (GO:0006955), positive regulation of bone mineralization (GO:0030501), defense response to virus (GO:0051607), inflammatory response (GO:0006954), positive regulation of osteoblast differentiation (GO:0045669), chemokine-mediated signaling pathway (GO:0070098), positive regulation of pathway-restricted SMAD protein phosphorylation (GO:0010862), positive regulation of phosphatidylinositol 3-kinase signaling (GO:0014068), positive regulation of protein kinase B signaling (GO:0015187), positive regulation of tumor necrosis factor ligand superfamily member 11 production (GO:0030309), endochondral bone morphogenesis (GO:0060350), negative regulation of chondrocyte proliferation (GO:1902731), positive regulation of cell proliferation (GO:00008294), positive regulation of cAMP metabolic process (GO:0030816), positive regulation of transcription from RNA polymerase II promoter (GO:0045944), positive regulation of cAMP-mediated signaling (GO:0043950), proteoglycan biosynthetic process (GO:0030166), angiogenesis (GO:0001525), transmembrane receptor protein serine/threonine kinase signaling pathway (GO:0007178), positive regulation of membrane protein ectodomain proteolysis (GO:0051044), positive regulation of cartilage development (GO:0061036), positive regulation of tyrosine phosphorylation of Stat1 protein (GO:0042511), regulation of cell proliferation (GO:0042127), positive regulation of leukocyte chemotaxis (GO:0002690), chondrocyte development (GO:0002063), negative regulation of smooth muscle cell proliferation (GO:0048662), regulation of insulin secretion (GO:0050796), positive regulation of release of sequestered calcium ion into cytosol (GO:00515281), lung alveolus development (GO:0048286), lymphocyte chemotaxis (GO:0048247), positive regulation of endothelial cell migration (GO:0010595), positive regulation of tumor necrosis factor production (GO:0032760), mesoderm formation (GO:0001707), blood vessel remodeling (GO:0001974), positive regulation of cell division (GO:0001781), positive regulation of epithelial cell proliferation (GO:00050679), chondrocyte differentiation (GO:0002062), negative regulation of angiogenesis (GO:0016525), stem cell population maintenance (GO:0019827), positive regulation of MAPK cascade (GO:0043410), positive regulation of T cell proliferation (GO:0042102), positive regulation of peptidyl-serine phosphorylation of STAT protein (GO:0033141), neutrophil chemotaxis (GO:0030593), cellular response to lipopolysaccharide (GO:0071222), glycoprotein binding (GO:0001948), growth factor activity (GO:0008083), chemokine activity (GO:0008009), receptor signaling protein serine/threonine kinase activity (GO:0004702), BMP receptor activity (GO:0098821), transmembrane receptor protein serine/threonine kinase activity (GO:0004675), cytokine activity (GO:0005125), CXCR chemokine receptor binding (GO:0045236), and protein tyrosine kinase activity (GO:0004713), respectively (Figure 2).

The 20 most significantly enriched pathways with the significant P-value threshold of $10^{-4}$ were related to extracellular space (TG, ADAMTS11, IGFBP6, BMP2, CXCL9, TGFB3, IGF1, IGF2, Dcn, Ccl4, Gas6, Cxcl10, Lep, Ace, Ifna1, Ifnb1, Ifng, Ace2, Tgfalpha, IGFBP3, IGFBP4, IGFBP5), extracellular region (Lep, Fgf19, Lif, Vve, Bdnf, IGFbp6, Ifg2, Fgf21, Ccl4, Gas6, Cxcl10), external side of plasma membrane (Ace, Ifng, Cxcl9, Ctl4a, Bmpr1a, Cxcl10), positive regulation of insulin-like growth factor receptor signal (IGFbp6, IGFbp3, IGFbp4, IGFbp5), positive regulation of cell proliferation (FGF2r2, Fgf19, Lif, Igf1, Tgfalpha, Fgf21, Bmpr1b, Cxcl10), positive regulation of ERK1 and ERK2 cascade (FGF2r2, Fgf19, Tek, Fgf21, Ccl4, Gas6), immune response (Lif, Cxcl9, Ctl4a, Tnfalpha1, Ccl4, Bmpr1a, Cxcl10), positive regulation of cell division (FGF2r2, TGFB3, Tgfalpha, IGF2), positive regulation of bone mineralization (Bmp2r2, TGFB3, Bmpr1b, Bmpr1a), positive regulation of epithelial cell proliferation (FGF2r2, Igf1, Tgfalpha, Bmpr1a), regulation of cell growth (IGFbp6, IGFbp3, IGFbp4, IGFbp5), positive regulation of osteoblast differentiation (Bmp2r2, Bmpr1b, Bmpr1a), proteoglycan biosynthetic process (Bmp2r2, Igf1, Bmpr1b), positive regulation of phosphatidylinositol 3-kinase signaling (Lep, Tek, Igf1, Dcn), positive regulation of protein kinase B signaling (Lep, Tek, Adaptam8, Gas6), defense response to virus (Ifna1, Ifnb1, Ifng, Cxcl9, Cxcl10), glycoprotein binding (Vve Ace2, Tgfa, Itgb2, Bmpr1b, Bmpr1a), growth factor activity (Lep, Bdnf, Tgfb3, Igf1, Tgfalpha, IGF2), insulin-like growth factor I binding (IGFbp6, IGFbp3, IGFbp4, IGFbp5), insulin-like growth factor II binding (IGFbp6, IGFbp3, IGFbp4, IGFbp5) (Table 2).

**Networks on the Whole Interactome of vitOrgan Cytokines**

The networks of DAVID analysis on whole vitOrgan cytokines are shown in Figure 2. The thickness of the line represents the strength of the correlation, and the thicker lines demonstrated the stronger of the interaction. Networks with top score (Figure 3) included proteins involved in chemokine-mediated signaling pathway, immune response and inflammatory response et al. including Ifn-β1, Ccl8, TnfαP3, Cxcl6, Ccl25, Tnfsf12, Tnfsf13b, Ccl40gl6, Pdcd1, Ifn-α1, Ccl4, Cxcl9, Cxcl5, Ccl7, Il1b, Cxcl10, Ccl5, Il15, Il2, Il3, Ctl4a, Cxcl12, Ifn-γ, Il10, Ccl2, Il17a, Il4, Il13, Tlr4, Il1b, Vegfa, Lep, Kitlg, Fgf1, Lif, Mmp3, Cds6, Mmp2, Notch1, Fgf2, Bdnf, Pecam1, Tek. The degree of a node indicates the number of links connected to a vertex. High degree nodes are the most relevant ones in interactome networks, as they represent the fulcrum of multiple signaling pathways, and their positive/negative modulation could result in alterations of the activities of their likely interactors. Sixteen main high-degree
nodes were present in this network, namely CXCL9, CCL4, CXCL5, IL18, CXCL10, CCL5, IL17A, IL4, CCL2, IL10, IFN-γ, CXCL12, VEGFA, MMP2, FGF2, IL13, CTLA4, linked to proteins involved in chemokine-mediated signaling pathway and cell proliferation.

Figure 2: Gene ontology (GO) classification of cytokines in vitOrgan.
Gene ontology (GO) and KEGG pathway classification of target genes. The x-axis shows the GO categories and y-axis shows the target genes. The x-axis shows the P-value and y-axis shows the diverse biological functions of target genes according to the GO categories.

Figure 3: The network analysis on whole vitOrgan cytokines
Grey nodes, proteins from the dataset having a match within the database; white nodes, proteins from the database that were not identified (if present) upon the experimental phase; grey edges, interactions within a network; continuous line edge, direct interaction; interrupted line edge, indirect interaction.
Vitorgan Cytokine-Cytokine Receptor Interaction

KEGG pathway is a collection of manually drawn pathway maps representing our knowledge on the molecular interaction, reaction and relation networks including metabolism, genetic information processing, environmental information processing, cellular processes, organismal systems, human diseases and drug development. Corresponding pathways can be predicted by KEGG analysis. Design and realization of software of vitOrgan cytokines analysis for evaluation is based on R Language and p value Cutoff = 0.05. We present a visualizing and integrating pathways using pathview package and demonstrated an interaction pathway which involved in as much more molecule of vitOrgan as possible.

This interaction showed 68 cytokines of vitOrgan and the red box labeled molecular denoted up-regulated cytokines, relating with chemokines (CCL25, CCL4,CCL4L1, CCL4L2, CCL17, CCL5, CCL8, CCL16, CCL7, CCL2, CCL12), CXC subfamily (CXCL5, CXCL6, CXCL9, CXCL10, CXCL12), the class I helical cytokines (IL-2, IL-4, IL15, IL3, IL4, IL13,IL27A, LIF), the class II helical cytokines(IL10, IL22, IFNA, IFNBI, IFNG), IL1-like cytokines(IL1B, IL1F5, IL1B, IL17-like cytokines(IL17A, IL17B, IL17C, IL17RA), TNF family (DCR3, XEDAR, TWEAK, GITR, BCMA, CD40LG) and TGFβ family (TGFB3, BMPR2, BMPR1A, BMP4, BMPR1B, BMP5) (Figure 4). The results provide brief information on the possible mechanisms of cytokines from vitOrgan that are in effect for the therapy through data analysis.

**Figure 4:** The cytokine-cytokine receptor interaction of KEGG view on gene data with compound data on a global and overview map. Red box labeled molecular are related to up-regulated proteins, while green box labeled molecular are related to down-regulated proteins.

**Discussion**

In this study, we focused on understanding the cytokine composition of concentrated vitOrgan injection solution by using cytokine antibody array. As a result, a list of 123 molecules was found in the concentrated injection solution. To our knowledge, this is by far the most extensive inventory of molecules from vitOrgan reported in the field. We have learned the types of molecules exist in vitOrgan and the types of cellular signaling pathways that these molecules are intensively involved in. The results can help us anticipate the molecular mechanism related to the treatment of vitOrgan and can further provide theoretical guidance for potential clinical applications. On the other hand, these cytokines from vitOrgan may also participate in the repair and metabolism of organs. Potential targeted organs include:

(i) Thyroid (Thyroglobulin 533.43 pg/ml present at top ten high concentrations in vitOrgan);
(ii) Brain (BDNF);
(iii) Bone (BMPRIA 557.66 pg/ml, present at top ten high concentrations in vitOrgan; BMP-4, BMP-5, BMPRIB, BMPR2);
(iv) Reproductive tract (Testican 2) (Figure 1) and (Table 1)[16]. Nevertheless, most cytokines of vitOrgan have been described acting on the whole body (Table 2), which suggest it may contribute to the homeostasis of the entire human physiological system.

The three molecules with the highest concentrations among cytokines in vitOrgan were related in these 20 most significantly...
enriched pathways including BMPRIA, IGFBP-3 and IGFBP-4. BMPRIA was involved in four most enriched pathways of external side of plasma membrane, immune response, positive regulation of bone mineralization and positive regulation of osteoblast differentiation (Figure 2). IGFBP-3 and IGFBP-4 together take part in four most enriched pathways of extracellular space, positive regulation of insulin-like growth factor receptor signal, regulation of cell growth and insulin-like growth factor 1 binding. In the case of IGFBP5 has been suggested that they may regulate IGFs functions in promoting cell proliferation/differentiation, because they:

(i) Act as transport proteins in plasma and control the efflux of IGFs from the vascular space;
(ii) Prolong the IGFs half-lives and regulate their metabolic clearance;
(iii) Help coordinating tissue- and cell type-specific localization of IGFs; and
(iv) Directly modulate interaction of IGFs with their receptors and thereby indirectly control its biological actions [16,18].

Therefore, the high concentrations of cytokines were related to a series of cell and molecular functional activities and would play an important role in life and health maintenance. There are 93 molecules in vitOrgan resembles those in milk proteome compiling of 573 entries [16]. These molecules are mainly involved in six network pathways, including

(i) Cell cycle, cancer, respiratory disease (VEGF, FGF-21, VEGF_R2, EEF1G, EGF, bFGF, APGF, FGFR4, FGFR7, FGFR9, FGFR17, FGFR2);
(ii) Lipid metabolism, molecular transport, small molecule biochemistry (TGFβ1, TGFβ2, TGFβ3, THBS1, LEP, BTC, EGRF, TGF-β2, IGFBP3, IGFBP1, IGFBP2, IGFBP4, IGFBP5, IGFBP6, IGFBP7);
(iii) Hematological system development and function, immune cell trafficking, inflammatory response (CXCL1, CXCL10, CXCL9, CCL4, ENA78, MMP-1, SDF-1, CX3CL1, CXCL11, CXCL12, CXCL13, CXCR3, GCP-2);
(iv) Carbohydrate metabolism, small molecule biochemistry, inflammatory response (IL-1 R3, CXCL1, IL-1R-1, IL-1R-2, IL-1RL1, IL-2Ra, IL-5Ra, IL-6R, IL-2RB, IL-1Ra, CD44);
(v) Cellular development, cell death, hematological system development and function (GIFTR, IL-17C, IL-21R, TLR4, IFNβ, IL-1, IL-10, IFN-α, IFN-γ, IL-17A, IL-18, IL-2, IL-4, IL-15, IL-13, IL-1 IS, IL-21, IL-3, IL-5, IL-7, IL8, IL-9, IL-11, IL12B, IL16, IFN-γ, Fas(TNFRSF6), NGF-R, Osteoprotegerin, TRAIL R3, TRAIL R4);
(vi) Cardiovascular system development and function, cellular development, skeletal and muscular system development and function (PDGFRb, PDGF AA, PECAM-1, PDGF Rα, PDGF Rβ, TIMP-1, TIMP-2, TIMP3, PDGFR, PDGFRB) [16,19] (Figures 3 and 4).

Table: Enriched pathways of vitOrgan and milk proteome

| Pathway                        | VitOrgan | Milk |
|--------------------------------|----------|------|
| Cellular development           | 40%      | 30%  |
| Immune response                | 35%      | 25%  |
| Hematological system development and function | 25% | 15% |
| Lipid metabolism               | 20%      | 10%  |
| Carbohydrate metabolism        | 15%      | 5%   |
| Inflammation                   | 10%      | 5%   |

Six pathways that shared ten cytokines between vitOrgan and bovine milk were listed above (i-vi) while the others with less shared cytokines were not listed. Because of such relevance, vitOrgan may help repair the damage to tissues and organs resulting from aging and environmental hazard, similar to the molecular mechanisms of bovine milk [16]. It would be helpful to study natural medicine in a scientific and systematic fashion to unveil the underlying mechanisms, which would point in the right development direction of natural medicine and complementary and alternative medicine in the future. Furthermore, the results of such a large biomedical data analysis require further clinical evaluation.

As we known live cell therapy (LCT) is an alternative treatment without medical evidence of effectiveness, which is through online marketing worldwide. It was reported the intramuscular injections of cell suspensions from fetal sheep, including young rams and pregnant ewes, were injected to human recipients for rejuvenation (anti-aging) and other ailments with positive feedback [20]. Apart from Germany recipients, medical tourists from North America and Asia travel to Germany to receive injections. Although several incidents were reported that LCT was the possible cause for the Q fever in Canada, Germany and the United States, vitOrgan have not experienced the issue. When working with vitOrgan, the health practitioners worldwide should pay more attention to the potential risk factors for LCT-induced Q fever [21,22].

Altogether, this study focused on the cytokines involved in vitOrgan and is by far the first investigation about the molecular composition of vitOrgan injection solution. We have found 123 cytokines using human array and 91 important signal pathways associated with development, proliferation, differentiation, immunology and metabolism. Moreover, a preliminary map of vitOrgan proteins interactome was also constructed, which can greatly help understand the mechanism of vitOrgan. With more in-depth knowledge of vitOrgan, clinician can put it into more targeted clinical applications and popularize the use of vitOrgan in the world.

### Reference

1. Robyn MP, Newman AP, Amato M, Walawander M, Kothe C, et al. (2015) Q Fever Outbreak Among Travelers to Germany Who Received Live Cell Therapy—United States and Canada, 2014. MMWR Morb Mortal Wkly Rep 64(38):1071-1072.
2. Rietschel HG (1953) Niehans fresh cell therapy. Medizinische 10(41): 1326-1329.
3. Koch E (1955) Historical dates concerning Niehans fresh cell therapy and critical remarks on some priority claims. Hippokrates 26(20): 609-613.
4. Pischinger A (1955) Fresh cell therapy; critical remarks on the theory and nature of Niehans’ fresh cell therapy. Wien Med Wochenschr 105(46): 952-957.
5. Jores A (1955) Critical comments on Niehans cellular therapy and on the methods of outsiders in medicine. Hippokrates 26(7): 206-209.
6. Brauch F (1956) Fresh-cell therapy. Dtsch Med Wochenschr 81(27): 1086-1088.

7. Bohl J, Goebel HH, Pötsch L, Eisinger W, Walther G, et al. (1989) Complications following cell therapy. Z Rechtsmed 103(1):1-20.

8. Heiss F (1991) Allergiebehandlung mit der Gegensensibilisierung (ALLERGOSTOP). Erfahrungsheilkunde 40(8): 510-513.

9. Dutta S, Singh G, Sreepith S, Mamidi MK, Husin JM, et al. (2013) Cell therapy: the final frontier for treatment of neurological diseases. CNS Neurosci Ther 19(1): 5-11.

10. Verbruggen S, Luining D, van Essen A, Post MJ (2018) Bovine myoblast cell production in a microcarriers-based system. Cytotechnology 70(2): 503-512.

11. Schooltink H, Rose John S (2002) Cytokines as therapeutic drugs. J Interferon Cytokine Res 22(5): 505-516.

12. Azodi S, Jacobson S (2016) Cytokine Therapies in Neurological Disease. Neurotherapeutics 13(3): 555-561.

13. Holdsworth SR, Gan PY (2015) Cytokines: Names and Numbers You Should Care About. Clin J Am Soc Nephrol 10(12): 2243-2254.

14. Belardelli F, Ferrantini M (2002) Cytokines as a link between innate and adaptive antitumor immunity. Trends Immunol 23(4): 201-208.

15. Olesen CM, Coskun M, Peyrin Biroulet L, Nielsen OH (2016) Mechanisms behind efficacy of tumor necrosis factor inhibitors in inflammatory bowel diseases. Pharmacol Ther 159: 110-119.

16. D Alessandro A, Zolla L, Scaloni A (2011) The bovine milk proteome: cherishing, nourishing and fostering molecular complexity. An interactomics and functional overview. Mol Biosyst 7(3): 579-597.

17. Shang Z, Li H (2017) Altered expression of four miRNA (miR-1238-3p, miR-202-3p, miR-630 and miR-766-3p) and their potential targets in peripheral blood from vitiligo patients. J Dermatol 44(10): 1138-1144.

18. Rajaram S, Baylink DJ, Mohan S (1997) Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions. Endocr Rev 18(6): 801-831.

19. Zhou J, Li F (2014) Potential pharmacokinetic interactions of therapeutic cytokines or cytokine modulators on small-molecule drugs: mechanistic understanding via studies using in vitro systems. Drug Metabol Drug Interact 29(1): 17-28.

20. George M, Reich A, Cussler K, Jehl H, Burckhardt F (2017) Live Cell Therapy as Potential Risk Factor for Q Fever. Emerg Infect Dis 23(7): 1210-1212.

21. Robyn MP, Newman AP, Amato M, Walawander M, Kothe C, et al. (2015) Q Fever Outbreak Among Travelers to Germany Who Received Live Cell Therapy—United States and Canada, 2014. MMWR Morb Mortal Wkly Rep 64(38): 1071-1073.

22. Robyn MP, Newman AP, Amato M, Walawander M, Kothe C, et al. (2015) Q fever outbreak among travelers to Germany associated with live cell therapy—United States and Canada, 2014. a co-publication. Can Commun Dis Rep 41(10): 223-226.

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