Searching the literature for proteins facilitates the identification of biological processes, if advanced methods of analysis are linked: a case study on microgravity-caused changes in cells

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\textbf{ABSTRACT}

\textbf{Background:} More than one hundred reports were published about the characterization of cells from malignant and healthy tissues, as well as of endothelial cells and stem cells exposed to microgravity conditions.

\textbf{Methods:} We retrieved publications about microgravity related studies on each type of cells, extracted the proteins mentioned therein and analyzed them aiming to identify biological processes affected by microgravity culture conditions.

\textbf{Results:} The analysis revealed 66 different biological processes, 19 of them were always detected when papers about the four types of cells were analyzed.

\textbf{Conclusion:} Since a response to the removal of gravity is common to the different cell types, some of the 19 biological processes could play a role in cellular adaption to microgravity. Applying computer programs, to extract and analyze proteins and genes mentioned in publications becomes essential for scientists interested to get an overview of the rapidly growing fields of gravitational biology and space medicine.

\section{1. Introduction}

Since more than 30 years, various types of human cells have been exposed to real or simulated microgravity conditions [1]. A number of observations on these cells clearly indicated that the lack of gravity, i.e. sedimentation, causes alterations of cells and their behavior [2–4]. An outstanding change of behavior is the switch from a two-dimensional to a three-dimensional (3D) growth [5]. This means that cells from human tissues, growing as monolayers under static 1-g conditions \textit{in vitro}, detach at least partially from the bottom of a culture flask and form 3D cell aggregates, suspended in the culture medium, when they are exposed to simulated or real microgravity. Endothelial cells (EA.hy926 cell line) [6], for example, may be cultured as monolayer under normal gravity for 3 weeks (Figure 1(a)). When cultured on a ground-based facility like the random positioning machine (RPM) [1], the cell population separates in cells which remain adherent, but show elongated fibers and stress fibers (Figure1(b)) and in cells which detach from the bottom of the culture dish and assemble to tube-like structures (Figure 1(c)). As a rule, these 3D cell aggregates become visible after 1–7 days of sedimentation prevention [7–10]. Afterward, they continue to grow up to 4 weeks.

The appearance of the aggregates varies depending on the type of cells studied. When cancer cells are cultured under microgravity, spheres or spheroids can very often be seen floating in the culture medium [7,9], while MCF-7 breast cancer cells may also form duct-like structures [11]. But when cells from healthy tissues are grown after annulling gravity, the shape of the aggregates resembles the structures of the organs from which the cells have been derived [9,10,12].

Although alternative methods of challenging single cells to form tumor or tissue-like aggregates are known, the microgravity-dependent method becomes more and more interesting, because it does not require scaffolds, which would influence the application of the tissue-engineered cell aggregates [5]. Currently, two options of application are discussed. In case the spheroids consist of cancer cells, they may be used as \textit{in vitro} systems to test antitumor strategies [13]. This is an advantage because these systems resemble the \textit{in vivo} situation much more than cancer cells grown in monolayers, but they are not as complex as natural tumors. If the spheroids consist of cells from healthy tissue like endothelial cells or chondrocytes, they appear to be suitable to engineer small vessels or pieces of cartilage [9,10]. Currently, \textit{in vitro} engineered 3D cell aggregates are used for research purposes only, but it is expected that cell structures suitable for transplantation purposes will be obtained in the future. It is still a problem that a number of genes and proteins, which are not directly involved in the
formation of 3D aggregates, are differently expressed [2,14]. These alterations may generate cell types that could be inapt for drug tests or transplantation. Therefore, the emphasis here is on alterations of cells in result to microgravity-dependent upregulation or downregulation of genes and their corresponding proteins.

During the last two decades, a number of scientists investigated changes in gene expression and amounts of proteins in various cell types exposed to microgravity. For this purpose, various methods of annulling gravity were applied. Cells were sent into orbit and cultured either on the International Space Station or on unmanned spaceships circuiting the Earth for a week or two [15–17]. In addition, cells were taken along on parabola-flying planes [18]. As these methods of generating weightless environments are extremely expensive, the researchers exposed the cells to devices preventing cell sedimentation under normal 1-g laboratory conditions. These instruments are called ground-based facilities. They include the RPM, the rotating-wall vessel, or the 2D clinostat (CN) [1]. After exposure to the RPM, cellular proteins were investigated by Western blotting and/or mass spectrometry [19], while the gene expression was determined by qPCR [20]. This way, substantial knowledge about the respective role of a number of proteins or genes was accumulated. However, little is known right now about their interactions or involvement in pathways of different biological processes.

In order to examine the interplay of single proteins, we collected publications about the research explained above and pooled the manuscripts according to the cell types described therein. Four different groups were defined, which included studies on cancer cells [5,7,8,15,17,21–46], on stem cells [2,47–71], on endothelial cells, [9,20,72–94] or on other healthy tissue cells [10,18,95–119], respectively. The characteristics of these cell groups were described in several publications referenced by [1,120–123].

The four groups of manuscripts were analyzed extracting proteins and genes described in the manuscripts of each group together, in order to obtain more information from recent publications about the influence that microgravity-dependent cellular alterations of gene expression patterns and protein contents has on the behavior and differentiation status of cells. Using the new methods of data mining and Pathway Studio analysis, we identified biological processes, which we expected to be involved in the signaling during cell migration, proliferation, or in organizing the protein structures of the extracellular space [124,125]. But to our surprise, the method revealed unexpected biological processes, such as responses to organic cyclic compounds or drugs. Hence, this review may draw attention to microgravity-dependent mechanisms not previously noticed.

2. Methods

2.1. Visualization of F-actin

F-actin was visualized by rhodamine-phalloidin staining (Molecular Probes, Eugene, OR, USA), and the nuclei were stained with Hoechst 33342 (Molecular Probes). The method was published previously in detail [7,33,95].

2.2. Collection of publications

Literature citations, which pointed to molecular biological studies on human tissue cells exposed to real or simulated microgravity were retrieved from the Web of Science (Thompson Reuters, Philadelphia, PA, USA), Scopus (Elsevier, Amsterdam, The Netherlands), and from recent papers listed in PubMed (https://www.ncbi.nlm.nih.gov/pubmed). After de-duplication, the full manuscripts were purchased from the various publishers and divided into four groups according to the cell type described therein.

2.3. Extraction and analysis of described proteins

MedScan Reader v6 and Pathway Studio v11 were purchased from Elsevier Research Solutions, Amsterdam, the Netherlands [126,127]. Using the Elsevier MedScan Reader, sentences were identified in the manuscripts of each group separately. The sentences were selected when proteins were mentioned within in context of cellular exposure to real or simulated microgravity. Then, the gene names were downloaded and transferred to SwissProt numbers, which in turn were entered in the Elsevier Pathway Studio. With the help of this program, biological processes, in which the collected proteins were enriched, were searched. It was also used to design the interaction network of the proteins that positively regulate cell migration (Figure 2).

3. Results and discussion

Our literature searches revealed 109 manuscripts listed in WoS or Scopus, which indicated that several cell types from various
tissues were investigated in recent years in regard to their molecular reactions on microgravity exposure. We divided the manuscripts into four groups (Table 1). One group (31 manuscripts) included studies on cancer cells [5,7,8,15,17,21–46]; a second one (26 manuscripts) studies on stem cells [2,47–71]. The third group (25 manuscripts) consisted of studies on endothelial cells [9,20,72–94] and the fourth group (27 manuscripts) of studies on other healthy tissue cells [10,18,95–119], like chondrocytes or hepatocytes (Table 1). The manuscripts in each group were analyzed using the MedScan Reader. The program detected 360 (#1), 247 (#2), 293 (#3), and 237 (#4) proteins named in at least one relevant sentence or 154 (#1), 111 (#2), 104 (#3), and 110 (#4) proteins named at least in three sentences (Table 1). The total number of proteins counted over all four groups was 1137. A great number of proteins were identified as not only part of one group; therefore, 769 different proteins were ultimately identified.

After the gene names coding for each identified protein were exported into Excel sheets, the accompanying SwissProt numbers were determined via a search at http://www.expasy.org and added to each respective protein. These numbers were uploaded into the Pathway Studio program in groups of 360 (#1), 247 (#2), 293 (#3), and 237 (#4) SwissProt numbers. Then, the Pathway Studio program was utilized to find ‘Pathways/Groups Enriched with Selected Entities’.

Table 2 shows a summary of the results obtained by the Pathway Studio analyses on the four protein groups mentioned above. In total, 66 types of biological processes were indicated in which proteins, found in the 109 analyzed manuscripts, were enriched. Proteins found in each group of the manuscripts contribute in 19 of these biological processes. Proteins described in three groups of the manuscripts were enriched in six biological processes. While proteins described in two groups of the manuscripts participated in 14 biological processes, 27 biological processes appear to be rather cell type specific, because each of them was found analyzing the proteins described in only one group of manuscripts, respectively.

Microgravity-dependent gene and protein alterations as well as the formation of 3D cell aggregates during exposure to real or simulated microgravity are common features of the cell types described in the four groups of manuscripts. Therefore, it appears reasonable to assume that at least some of the 19 biological processes, which emerge each time when proteins of one group are analyzed (Table 2), may be important in tissue formation or adaptation to the
microgravity environment. On the other hand, cell processes in which proteins participate, which attracted attention when one of the cell types was investigated, appear to be characteristic for the respective kind of cells. For example, both organ morphogenesis and organ regeneration are capabilities of stem cells [128,129], while ossification can be

Table 2. Overview on biological processes of which member proteins were described in the manuscripts selected. The percentages are indicated, at which the proteins found in the different groups of manuscripts cover all proteins involved in the respective biological processes.

| Biological process | Cancer cells | Stem cells | Endothelial cells | Other tissue cells |
|--------------------|--------------|------------|------------------|-------------------|
| Cell adhesion      | 8            | 6          | 7                | 5                 |
| Negative regulation of apoptotic process | 11          | 7          | 8                | 7                 |
| Negative regulation of cell proliferation | 10          | 8          | 9                | 8                 |
| Blood coagulation  | 10           | 8          | 9                | 10                |
| Positive regulation of cell proliferation | 12          | 8          | 11               | 8                 |
| Positive regulation of gene expression | 13          | 11         | 11               | 9                 |
| Response to drug   | 14           | 9          | 11               | 10                |
| Angiogenesis       | 13           | 9          | 17               | 12                |
| Response to lipopolysaccharide | 15          | 3          | 19               | 10                |
| Extracellular matrix organization | 14          | 12         | 15               | 14                |
| Response to organic cyclic compound | 15          | 12         | 16               | 13                |
| Aging              | 17           | 11         | 17               | 12                |
| Platelet activation| 19           | 11         | 15               | 14                |
| Response to hypoxia| 19           | 11         | 17               | 13                |
| Positive regulation of cell migration | 18          | 13         | 19               | 14                |
| Response to estradiol | 19          | 15         | 19               | 13                |
| Wound healing      | 21           | 16         | 22               | 16                |
| Cellular response to mechanical stimulus | 25          | 18         | 22               | 23                |
| Positive regulation of apoptotic process | 10          | 10         | 9                | 9                 |
| Positive regulation of transcription from RNA polymerase II promoter | 10          | 6          | 5                | 5                 |
| Positive regulation of transcription, DNA-templated | 7            | 8          | 7                | 7                 |
| Response to organic substance | 16          | 13         | 20               | 17                |
| Response to ethanol | 18           | 13         | 17               | 17                |
| Cellular response to interleukin-1 | 29           | 31         | 18               | 18                |
| Cellular response to organic cyclic compound | 26          | 18         | 22               | 18                |
| Extracellular matrix disassembly | 6           | 6          | 18               | 18                |
| Innate immune response | 6            | 6          | 18               | 18                |
| Ossification       | 22           | 19         | 18               | 18                |
| Platelet degranulation | 26          | 19         | 18               | 18                |
| Positive regulation of angio genesis | 18          | 18         | 22               | 20                |
| Positive regulation of peptidyl-serine phosphorylation | 22          | 22         | 20               | 20                |
| Positive regulation of smooth muscle cell proliferation | 28          | 35         | 35               | 35                |
| Response to cytokine | 26           | 29         |                  | 29                |
| Response to estrogen | 17           | 21         | 18               | 16                |
| Response to glucocorticoid | 37          | 35         |                  | 35                |
| Vascular endothelial growth factor receptor signaling pathway | 16          | 35         | 35               | 35                |
| Positive regulation of angiogenesis | 10          | 10         | 9                | 9                 |
| Axon guidance      | 8            |            | 18               | 18                |
| Cartilage development | 12          | 20         |                  | 20                |
| Cell-matrix adhesion | 18          |            |                  | 18                |
| Epidermal growth factor receptor signaling pathway | 14          |            |                  | 14                |
| In utero embryonic development | 8            |            |                  | 8                 |
| Integron-mediated signaling pathway | 18          |            |                  | 18                |
| Leukocyte migration | 17           |            |                  | 17                |
| Lipopolysaccharide-mediated signaling pathway | 48          |            |                  | 48                |
| Multicellular organism development | 4           |            |                  | 4                 |
| Negative regulation of transcription from RNA polymerase II promoter | 5            |            |                  | 5                 |
| Neurotrophin TRK receptor signaling pathway | 12          |            |                  | 12                |
| Notch signaling pathway | 18          |            |                  | 18                |
| Organ morphogenesis | 13           |            |                  | 13                |
| Organ regeneration | 19           |            |                  | 19                |
| Osteoblast differentiation | 16          |            |                  | 16                |
| Positive regulation of blood vessel endothelial cell migration | 61          |            |                  | 61                |
| Positive regulation of epithelial cell proliferation | 21          |            |                  | 21                |
| Positive regulation of epithelial to mesenchymal transition | 40          |            |                  | 40                |
| Positive regulation of ERK1 and ERK2 cascade | 14          |            |                  | 14                |
| Positive regulation of MAPK cascade | 18          |            |                  | 18                |
| Regulation of cell proliferation | 14          |            |                  | 14                |
| Signal transduction | 4            |            |                  | 4                 |
| Skeletal system development | 14          |            |                  | 14                |
| Transforming growth factor beta receptor signaling pathway | 13          |            |                  | 13                |
The described new method of reviewing literature by advanced techniques of data collection and subsequent pathway analysis may draw attention to new research targets and help to promote specific projects. Of course, similar results as shown here could possibly be achieved by collecting the genes and proteins manually from the considered number of manuscripts. However, the time required would be enormous. In addition, it appears virtually impossible to retrieve biological processes, in which proteins of interest are taking part, from the literature without programs like the Pathway Studio and their underlying databases. Unfortunately, the programs available nowadays do not allow for the extraction of the given information in a paper about a protein together with the protein’s name, so that phenomena such as the time-dependent variation of proteins can be extracted from the publications in a direct and automated way.

5. Expert commentary

Research on the behavior of cells generated a tremendous number of reports. All these manuscripts contain a huge amount of detail information. Summaries of the manuscripts may be found in many traditional literature databases. But these only show titles, abstracts, and a few keywords of each manuscript. Therefore, up to now, personal reading of the underlying full papers has been necessary. This process is time-consuming. In addition, human insufficiencies often prevent a complete extract of the information given by the authors. Hence, computer programs suitable to extract the detailed information of whole manuscripts can help to gain a better overview on the results described by the community performing research on a topic such as gravitational biology or space medicine. This could contribute to avoid expensive and useless research on already known results. In addition, it facilitates a direct comparison of a high number of manuscripts and reveals information mentioned in a single publication only along the way. If such a by-the-way-information is found in many manuscripts, it could challenge researchers to take a closer look at the described phenomenon. Thus, it will be avoided that an important aspect of a scientific question is overlooked.

Furthermore, the assignment of proteins found in an experiment to biological processes needs the knowledge of all or a high number of proteins taking part in a defined biological process or signaling pathway. This knowledge cannot be gathered by a single team of researchers after each relevant experiment, because a comprehensive list for all entities of each process would have to be generated. Therefore, databases containing information about the interactions of proteins or genes were built up by companies or organizations [127,140]. Matching genes and proteins detected by a researcher in experiments [15,79] or by extracting literature against such databases adds tremendous value to each experiment or literature search.

The field of space research has expanded in recent years and will surely further increase in the near future. Therefore,
the knowledge about the behavior of cells under microgravity will continue to accumulate. In order to keep up with the increasing amount of information gathered in microgravity research, the application of computer programs, which help to extract and analyze proteins and genes detected in the various experiments, will become more and more necessary.

6. Five-year-view

Scientific literature is currently doubling in growth [141]. Therefore, computer programs like the ones described in this review will become more and more important in the next 5 years. Their dissemination will increase, as well as the numbers of fields of application. Under these conditions, more effort will be invested to improve the programs and their underlying databases. Hence, interaction databases of proteins and genes of more species than human, mouse, rat, and Arabidopsis thaliana may be available soon, each containing a reasonable number of entities. Also, the number of automatically analyzable substances and topics will expand.

Regarding the automatic detail extraction from manuscripts, improvements can also be expected. One can assume that not only proteins and genes will be extractable in 5 years, but also many other entities either alone or in combination. Therefore, these new techniques will become essential in many research fields, but also in microgravity research, which surely will continue to grow in the next few years.

Key issues

- OMICS investigations (Proteomics, Metabolomics, Genomics) are important in the field of Gravitational Biology and Space Research in order to understand the effects of microgravity on biological processes.
- Value may be added to the results of individual experiments, when the detected proteins, genes or metabolites are further analyzed using programs like the described Pathway Studio.
- A fast assigning of detected proteins to known biological processes appears advantageous for subsequent research.
- Like in many other research fields the knowledge accumulated in the field of Gravitational Biology and Space Research is tremendously increasing.
- It is difficult and time consuming to gather all detail information, which are described in single manuscripts, manually.
- Computer programs for mining literature will become more and more necessary for a scientist to keep up with the accumulating knowledge.
- If by-the-way results are found in many manuscripts, their importance could be recognized.
- If the biological process is known to which a detected protein belongs, it can better be decided whether it plays a role in cellular adaptation to microgravity.

Declaration of interest

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