Brief Report

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MVsCarta: A protein database of matrix vesicles to aid understanding of biomineralization

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Summary **Matrix vesicles (MVs) are membranous nanovesicles released by chondrocytes, osteoblasts, and odontoblasts. They play a critical role in modulating mineralization. Here, we present a manually curated database of MV proteins, namely MVsCara to provide comprehensive information on MVs of protein components. In the current version, the database contains 2,713 proteins of six organisms identified in bone, cartilage, tooth tissues, and cells capable of producing a mineralized bone matrix. The MVsCarta database is now freely assessed at *http://bioinf.xmu.edu.cn/MVsCarta*. The search and browse methods were developed for better retrieval of data. In addition, bioinformatic tools like Gene Ontology (GO) analysis, network visualization and protein-protein interaction analysis were implemented for a functional understanding of MVs components. Similar database hasn't been reported yet. We believe that this free web-based database might serve as a useful repository to elucidate the novel function and regulation of MVs during mineralization, and to stimulate the advancement of MV studies.**

Keywords: Matrix vesicles, database, mineralization, proteomics

1. Introduction

Matrix vesicles (MVs) are small (20-200 nm) membrane particles bedded off from the plasma membrane of mineralizing chondrocytes, osteoblasts, and odontoblasts (*1*). MV-like particles have also been observed in the media of cultured mineralizing cells (MMV) or a number of ectopic calcifications such as vascular smooth muscle cells (VSMC-MV) (*2,3*). Usually, the MVs or MV-like particles are released into the pre-mineralized organic matrix prior to the onset of matrix mineralization, and act as a key constituent in both physiological and pathological mineralization. Current evidences support two possible mechanisms for MVs involving mineralization: first, they modulate the local Pi/Ppi ratio in the mineralizing matrix; secondly, they provide initial mineral nucleation sites for mineralization (*4,5*).

Although originated from the plasma membranes, the composition of MVs is different from that of the membranes they budded off from (*6*). The principle components of MV modulating mineralization are proteins and lipids. A panel of MVs proteins have been identified and functionally clarified including some enzymes [Alkaline phosphatase (TNAP), Phospho-1, Na+/K+ATPase, nucleotide pyrophosphatase/phosphodiesterase I-1 (NPP1/PC-1), matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-3 (MMP-3), matrix metalloproteinase-13 (MMP-13)], transport proteins (annexin 5, annexin 2, annexin 6, annexin 11, annexin 4, annexin 1, annexin 7, Pit 1, Pit 2), and integrin proteins (integrins β1, β5, αV, α11, α1, α3) (*4*). These proteins are shown to be linked to the two key functions of MV in modulating mineralization.

While progress has been made in the study of MVs,

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much still remains to be clarified. A comprehensive picture of MVs components is very helpful to ascertain the following key issues in biomineralization: how MVs are formed and regulated; how MVs participated in the physiological and pathological process. With the rapid development of proteomic tools, high-throughput analyses of MVs protein profiles are blossoming in recent years (*3,7-10*). Data on the MVs protein component have been generated. Given the value of the already generated large amounts of data and the likely increase of MVs studies in the future, a public compendium to store and retrieve such data is required.

Here, we constructed a web-based compendium of MVs, namely "MVsCarta" (*http://bioinf.xmu.edu.cn/MVsCarta*), to catalogue the MVs protein components. The information collected in MVsCarta was derived from exhaustive literature research of both proteomic and functional studies, then was further annotated manually. We believe that this free web-based community resource will aid researchers to better understand the role of MVs in mineralization and trigger new studies on MVs.

2. Materials and Methods

2.1. *Data Retrieval and preprocessing*

The information of martrix vesicles was mainly obtained by querying the PubMed literature database (*http://www.ncbi.nlm.nih.gov/pubmed/*) using the combinational keywords of "matrix vesicles" as well as either "mineralization" or "calcification". A further selection was undertaken to pick up the articles containing the high-throughput proteomic studies, individual validation and function studies on MVs proteins. Subsequently, manual information retrieval was performed to extract the information regarding isolation and/or validation of MVs and identification and/or validation of MVs proteins from the full texts or supplemental data. Before uploaded into the database, the data were further cleaned by removing the incomplete or ambiguous records, and integrated by adopting unique IDs. For instance, all the MV proteins were represented by the UniProt access number (UniProt AC). In addition, all the MV proteins were assigned into either type of MMV and Collagenase-Released Matrix Vesicles (CRMV) based on their extracellular forms.

2.2. *Database construction*

MVsCarta (version 1.0) was constructed on Red Hat Linux release 9 operating system and the data were managed by the Relational Database Management System (RDBMS) Oracle 10g. Friendly interfaces and search engines were designed using the JSP technology and run on a Tomcat server.

3. Results and Discussion

The MVsCarta provides three methods for data retrieval: QUICK SEARCH, ADVABCED SEARCH, and BROWSE. The quick search method on the homepage and other Web pages allows foolproof text search using any single complete or partial keyword of gene symbol, protein name, UniProt AC, MV type, gene ID or organism. Keyword combination and wild characters like "*, +" are not supported yet. The records that meet the query criteria will be listed in the ascending alphabet order of gene symbols. Empty input in the text field of the quick search form (which is not recommended) will respond the full list of MV genes. Clicking on the gene symbol will redirect the user to the detailed information page, where comprehensive information of the MV proteins is given in five sections when available. The first section is gene description, which demonstrates the basic information of the MV protein like gene symbol, Entrez gene ID, protein name, and UniProt AC. Cross-links to the Entrez gene database and the UniProt protein knowledge base are provided. In the section of experimental evidences, one or multiple experimental evidences of MV proteins are given, including the organism, sample, MV isolation and validation method, MV type (CRMV or MMV), protein identification and validation method, and reference. The third section provides functional annotation of MV protein that whether the protein is involved in the mineralization or calcification when available. While in the fourth section, sub-cellular localization of the MV protein is given. In the last section of "Bioinformatic Analysis", two hyperlinks to the Gene Ontology analysis and the Interaction analysis are provided for rapid functional analysis of the designated MV genes/proteins along with its protein sequence in fasta format. As an alternative solution, the MVsCarta database offers an advanced search method for accurate data retrieval that allows user to specify the keyword search under three optional categories of MV types, samples and organisms.

In addition to the keyword search methods, the MVsCarta also offers the browse method for direct access to the database. All the MV proteins were pre-assigned into several groups subject to one of the following four characteristic categories: organisms; sample materials (including 3 tissues and 3 cell types); MV types (MMV or CRMV); and gene symbol in the ascending alphabet order. The user is allowed to rapidly access a group of MV proteins by selecting one from above four categories. As an open-access database, the data of MVsCarta can be downloaded *via* the query form in the "DOWNLOAD" page. The query results are output in the tab-delimited text format.

To aid better understanding the functional characteristics of MV proteins, three bioinformatic tools were developed and implemented into the database. The "Gene Ontology (GO)" analysis allows retrieving

the gene ontology information by inputting a gene/protein name or its corresponding ID in the text fields of the query form. The "Interaction Analysis" provides visualization of protein interactions of the query MV protein with other proteins. The protein-protein interaction information was derived from the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database. The MV proteins in the interaction map are highlighted in blue. These two tools were also implemented into the detail information page in the section of "Bioinformatic Analysis" for convenient access. For better understanding the relations between MV proteins, a dynamic network analysis tool was implemented. User is asked to submit a list of MV proteins separated by semicolon *via* the query form. At least two proteins/genes are required for network analysis. These query MV proteins that may be linked together within one gene network or form several isolated clusters. The implemented network analysis tool is generally compatible with current popular web browsers. These analyses on MV components provide global insights into the mechanisms involved in MV biogenesis as well as the role of MVs in mineralization or calcification.

Currently, the MVsCarta comprises 2,713 entries, 2,391 unique genes and 19 sample materials. These MV proteins were mainly isolated from the mineralized cultured cells *in vitro* and the mineralized tissues *in vivo* (bone, cartilage, and tooth), involving six different organisms such as Homo sapiens, Mus musculus, Rattus norvegicus, Rabbit, Gallus gallus, and Bos taurus. The MVsCarta database is expected to update regularly when the data is available. To aid rapid update, an online submission form was designed in the "Feedback" as well for acceptance of user-submitted data.

In summary, MVsCarta is the first online resource that provides comprehensive information of MVs protein components. With the increasing studies on lipid composition of MVs become available, lipidomics data will be incorporated to MVsCarta in the future to better understand the important role of cellular lipid metabolism in biological mineralization. In addition, more analysis tools are desired to implement to explore the biological and functional significance of the MVs components such as predicting calcium-binding sites in MV proteins. Nevertheless, this database will serve as a useful repository to elucidate the novel function and regulation of MVs during mineralization, and to stimulate the advancement of MV studies.

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