AGINGDB: A DATABASE FOR OXIDATIVE STRESS AND CALORIE RESTRICTION IN THE STUDY OF AGING

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ABSTRACT

Aging can be characterized in all living organisms as the inevitable biological changes that occur with advancing age. The aging process is time-dependent and leads to functional declines and increased incidences of disease. The underlying pathophysiologic processes of aging may best be explained using several interacting biological processes: genomic activity, oxidative stress, and age-related disease processes, all of which modify the rate and progression of aging. In this report, we describe a database, termed AgingDB, used to retrieve information on the biomolecules known to be modulated during the aging process and by the life-prolonging action of caloric restriction (CR). To enhance the usefulness of AgingDB, we include data collected from studies of CR's anti-oxidative action on gene expression, oxidative stress, and many chronic age-related diseases. We organized AgingDB into two sections A) apoptosis and the various mitochondrial biomolecules that play a role in aging; B) nuclear transcription factors known to be sensitive to oxidative environment. AgingDB features an imagemap of biomolecular signal pathways and visualized information that includes protein-protein interactions of biomolecules. Authorized users can submit a new biomolecule or edit an existing biomolecule to reflect latest developments. By making available the most update information through AgingDB, we expect to assist researchers who are exploring the molecular basis of age-related changes modified by the life-prolonging action of CR. For the reader's convenience and accessibility, AgingDB is freely available at <http://agingdb.bio.pusan.ac.kr/>.

INTRODUCTION

Aging results in functional deterioration in all living organisms (1). Although the course of the aging process is influenced likely by genetic alterations, oxidative stress, and age-related disease (2), the complexity of aging-related biological changes, the pathogenesis of various chronic diseases, and the effects of oxidative stress-related physiological functional deterioration all need clearer molecular explanations to better understand their roles in the aging process as well as the anti-aging actions of caloric restriction (CR).

Molecular explanations are obtained typically through functional studies of key biomolecules and pathways involved in biological process such as inhibition, activation, and interaction (3). Therefore, we constructed a database, termed AgingDB, based on the biomolecules known to be involved in various cellular regulatory functions during the aging process, to which we added the signal pathways of these biomolecules. Specifically, AgingDB focuses on the key factors of oxidative stress and CR (2-4); antioxidant-related biomolecules such as super oxide dismutase (SOD), glutathione (GSH), and catalase (5); and the anti-aging action of CR.

To create visual representations of signal pathways and protein-protein interactions in AgingDB, we used a graph algorithm (6) and an image template from BioCarta (<http://www.biocarta.com>). The visualizations of molecules offered by AgingDB are expected to be extremely useful in understanding the cellular and molecular mechanisms underlying the modulation of the aging process. The information on oxidative stress and the anti-aging action of CR also should be helpful to researchers of aging.

Currently, human and rat data form the database. An extensive literature review was undertaken to identify biomolecules that have a role in aging. Moreover, detailed information on biomolecules, such as name, sequence, and domain were obtained for the primary database (7). AgingDB can employ a keyword search, linking to an imagemap and an automatic protein interaction layout. Results from a query are displayed in a relational table, with pertinent links to Websites such as Genbank, Pubmed, and OMIM. Imagemap and protein interaction layouts will prospect function of biomolecules in the cell.

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RESULTS

Keyword search

The keyword search page (Fig. 1) enables quick and simple searches for information on items contained in AgingDB. Text entered into the search window will be returned to requestor with exactly matched items and a report giving detailed information (Fig. 2). The search function also allows the user to specify search terms in individual fields. Pull-down menus are available for the following fields: 'Symbol/name, Species, Cellular location, Organ, Domain, Function, Interaction, DNA name, DNA accession number, Protein name, and Protein accession number-'

Imagemap Retrieval

AgingDB is divided into two imagemaps: A) redox-sensitive transcription factors (e.g., NF κ B, AP-1, and HIF-1) (Fig. 3); B) mitochondria-related apoptosis (either from oxidative stress or from death domain receptor) (Fig. 4). Within each category, many items are hyperlinked to other resources to give more information on the subject. Imagemaps include information on kinase, activators, inhibitors, homodimer, heterodimer, and CR effect. We made imagemaps using a Biocarta template. (<http://www.biocarta.com>). The Biocarta template is made by standard icons to facilitate in the understanding of complex biological pathways.

Protein interaction map

To explore the interactions of all biomolecules contained in the AgingDB, protein interaction maps are based on spring algorithm of graph theory (6). The biomolecules that interact with other biomolecules are divided into three groups, complex (combine, bind), activate (increase, reduce), inhibit (decrease, induce) in order to clear up relational attributes of complex aging-related biomolecules. (Fig. 5,6)

Data Submission

AgingDB gives users the ability to submit data in the reporting of a new biomolecule or in the editing an existing biomolecule. Researchers performing these tasks will be certified or identified by the database manager (aging@pusan.ac.kr) or the principal investigator (hyjung@pusan.ac.kr) prior to gaining access to AgingDB. The protein interaction map is updated automatically in real-time from the information entered by personnel certifying the data submission. The updating of imagemap data pertaining to redox sensitive factors and mitochondria-related apoptosis are manually performed, monthly. We encourage the submission of data. High quality information from many laboratory sources is of utmost importance in achieving the ultimate goals of AgingDB, to track the signal pathways associated with aging and to understand mechanisms of aging. The schema of AgingDB is showed Fig. 7.

DISCUSSION

The purpose of the AgingDB is to arrange aging-related biomolecules and their signal pathways into a common filing system, because aging-related biomolecular changes are diverse and complex phenomena. We

accomplished this initially by dividing complex processes into two major sections: A) apoptosis and the various mitochondrial biomolecules that play a role in aging comprise one imagemap; B) nuclear transcription factors known to be sensitive to oxidative environment comprise another. The data retrieved from this section can produce an imagemap that could be particularly helpful in visualizing one biomolecule's overall relationship with age-related changes.

A special feature of AgingDB is offer a user-friendly environment to simplify the understanding of signal pathways and protein interactions through the visualization of various biological interactions of the aging process. The protein-protein interaction map allows easy retrieval of key factors such as inhibitors, activators, and frequencies of interaction. For example, we easily can gain information about the signal pathway of redox sensitive transcription factors that NF κ B genes regulate, such as interleukin-6, interleukin-12, interleukin-8, TNF-, RANTES, ICAM, VCAM, E-selectin, iNOS, COX-2, c-IAP, Bcl-cl, c-myc, and cyclin D1. NF κ B dependently interacts with other proteins, such as IKK, I κ B α , Thioredoxin, and Redox factor 1. Through such retrieval, we also can see that this IAP inhibits four other apoptosis proteins, making it a possible key factor for anti-apoptosis.

Furthermore, a database compiled from studies using the calorie restriction (CR) paradigm are very important, because CR's life-prolonging effects are the accepted as the "gold standard" for the intervention in aging processes at this time. In this regard, AgingDB applies the unique nature of CR to provide insightful information that may elucidate the mechanisms of aging and CR's anti-aging actions. The data contained in AgingDB is carefully collected to include aging-related biomolecules; and protein interactions.

The next goal of AgingDB is the acquisition of data that is comprehensive as possible, such as literature mining information (Telemakus Project (<http://www.telemakus.net>), gene information (AGEID (8)), DNA chip information (9,10,11), protein interaction information (12), and integrated information that predicts aging-related protein interactions on targeted proteins. In the future, AgingDB may help find new biomarkers for the aging process and various aging-related signal pathways.

EXPERIMENTAL PROCEDURES

Data Acquisition

Genetic and protein sequence data were obtained primarily from GenBank (13) and SwissProt (14). The signal pathways for each gene or protein were obtained by a literature review of PubMed (13). All relevant literature was searched using the following keywords: "aging", "oxidative stress", "anti-aging", and "calorie restriction". A close review of each article for pertinent information regarding protein-protein interaction, modulating factors, and cellular locations of various processes were undertaken in relation to the keywords. This information was then categorized and stored in AgingDB. Genetic and protein data consisted of se-

quence, aging-related function, interaction information, and protein domain. We obtained the protein domain data from Pfam (15); this data is used to predict function of sequence. Information on biomolecules that may have a role in age-related diseases was obtained from the literature search and from *OMIM* (13).

Database Fields

AgingDB contains two tables. One (profile table) shows a detailed profile of the targeted gene or protein and its aging-related function. The other (sequence table) shows the sequence of the gene or protein. The profile table contains 14 fields, consisting of an ID (primary key), NCBI accession number, symbol, name, aging-associate feature (increase, decrease, CR), animal species, cellular location, organ/tissue, function, signal pathway, interaction (activation, inhibition), complexes, related diseases, and references. For example, the 'cellular location' fields of NF κ B 1(p50) are cytoplasm and nucleus. This indicates that NF κ B 1(p50) may exist in both cytoplasm and nucleus or that it may be translocated from one to the other. A detailed visual representation is supplied on the signal pathway map, where the translocation of the heterodimer of p50 and p65 from the cytoplasm to nucleus through the activation of IKK and MAPKinase can be seen clearly. Additionally, activity such as the role of NF κ B in regulating transcription factor genes, such as cyclooxygenase-2, inducible nitric oxide synthase, and proinflammatory cytokines can be seen. Therefore, detailed visual information is usually obtainable by activating an existing hot spot in the text file, which will link to the molecule diagram of interest.

The sequence table consists of 6 database fields: DNA accession number (NCBI), DNA name, DNA sequence, protein access number (NCBI), protein name, and protein sequence. DNA and the resulting protein are hyperlinked to NCBI (13). Available information from AgingDB can be categorized as shown in Table 1 of this manuscript.

Data Categories

AgingDB consists of three major categories: A) the total biomolecules contained in AgingDB; B) the biomolecules that are altered by aging; C) the biomolecules that are modulated by CR. Each biomolecule is stored in the database according to its aging-effect (increase / decrease / no change / blank). The meaning of 'blank' is that a measurement for the biomolecule's effect on aging does not exist. The effects of CR are also recorded (increase / decrease / no change). Currently, the number of biomolecules increasing with aging is 32, the number of biomolecules decreasing with aging is 7 and the number of biomolecules changing with CR is 28.

Information Technology

Data for AgingDB is stored and maintained using the relational database management system (RDBMS), MySQL 3.23.27 (<http://www.mysql.com>). For data retrieval, CGI (Common Gate Interface) technology using PERL (<http://www.perl.com>) was integrated into the system. For the visualization of biomolecule interaction, we used SVG (Scalable Vector Graphics) 1.0

version based on XML (eXtensible Markup Language). The SVG is a W3C (WWW Consortium) norm for 2D vectorial graphics on the Web. Access to AgingDB is freely available through the Internet at URL <http://agingdb.bio.pusan.ac.kr/>.

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ABBREVIATIONS

ROS: reactive oxygen species
NF κ B: nuclear factor kappa B
AP-1: activator protein-1
HIF-1: hypoxia inducible factor-1
CR: calorie restriction
I κ B α : inhibitory kappa B alpha
IKK: inhibitory kappa B kinase
MAPK: mitogen activated protein kinase
GSH: glutathione
TNF- α : tumor necrosis factor alpha
iNOS: inducible nitric oxide synthase
COX-2: cyclooxygenase-2

Figure 4: Imagemap for mitochondria-related apoptosis.

Figure 5: Total interactions of target biomolecule in AgingDB.

Node colors of protein interaction vary with the number of interactions with other nodes: red indicates seven interactions; reddish-yellow, six; pink, five; brown, four; green, three; yellow, two; and black, one.

Figure 6: Interaction of target protein. Users can view activator, inhibitor, and complex of target protein. Also a loop on the viewer indicates homodimer (e.g., BID, BAX, BAK)

Figure 7: AgingDB schema.

Table. 1: Number of items contained in AgingDB as of May 2003.

Object	Count	Object	Count
Signal pathway	5	Aging increase	32
Interaction	70	Aging decrease	7
Protein	68	Caloric restriction	28
Gene	41	Reference	126

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