ACBD: Database for Ascidian Chemical Genomics

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Abstract

Chemical biology approach enables us to understand the complex biological systems, using small molecules such as a specific activator or inhibitor of protein, a hormone-like inducer, or a neurotransmitter. When such approach is performed genome-widely, that research is especially called "chemical genomics". We are planning to make a new start of chemical genomics using one of chordate model animal, ascidian. As a first step, we constructed a database called ACBD (Ascidians Chemical Biology Database).

First, we reviewed and annotated past articles which describe the uses of small chemicals in the field of ascidians biology. In ACBD, chemical information and effects on ascidian are manually extracted from more than 900 articles in PubMed database from 1964 to 2010. ACBD is free and open to the public on the web. ACBD has two main parts. One part consists of information about already-used chemicals to ascidians. This part is based on the record of already-published articles. In this part, we realized that more than 351 kinds of chemicals were applied for ascidian and that more than 399 kinds of chemicals were isolated from 120 kinds of tunicates!

The other part consists of "not-yet-used chemicals" information. Although the total number of *Ciona* protein model (KH model, Satou et al., 2008) is said to be 24,025, only 199 kinds of KH models have ever modified by chemical compounds. To know the number of potentially modifiable target proteins by currently available chemicals, we searched KH model against amino acid sequences of drug targets of chemicals recorded in DrugBank (Wishart DS et al., 2008). As a result, we found that 1,862 KH *Ciona* models might be modifiable by using commercially available chemicals. Thus, we can say that 7.8% of *Ciona* models have a potential to be modified their functions by chemicals. These information in ACBD are usefull for researchers interested in drug screening as well as chemical genomics. In near future, we are planning to integrate ACBD into CIPRO and ANISEED.

Introduction

In the field of chemical biology, traditionally vertebrates (rats, mice, and zebrafish) are preferred model organisms. However, these animals seem not to be appropriate for chemical genomics which requires a lot of times applying chemicals to model organisms and observing these results. We believe ascidians are preferred model organism in this study field. This is because, comparing with other model organisms, ascidians have lower cost and shorter developmental time. These characteristics make ascidians suitable for model animal for chemical genomics as well as whole-animal drug screening.

As a first step for starting chemical genomics using ascidians, we are constructing a database called ACBD. In the future, this database would be of a great help for gaining experimental information in ascidian chemical genomics.

Fig. 1 shows the overview of ACBD. The red-colored part is "Already-used chemicals part." This part is based on articles from PubMed. These articles are all describing experiments closely related to both chemicals and ascidians. We defined "chemicals" as small molecules sometimes including organic compounds, inorganic compounds, sugar, metal complex, metal-organic compounds, nonstructural materials and nucleic acid. We excluded kinds of proteins and enzymes, unless "cas" compound ID number was added. We retrieved around 900 articles from PubMed (using search terms as follows: Inhibitor, Activator, Treatment, Detailed chemical names × ascidians, pharmacology, pharmacological).

Through the process of constructing already-used chemicals part, we could review the history of chemical biology using ascidians.

Figure 1 Overview of ACBD

Result

Ascidians vs Chemicals DB, ACBD

Figure 2 The Screenshot of ACBD

This is the interface of ACBD. It has already been updated on the web. From more than 900 articles, we realized 750 (used 351+ isolated 399) kinds of chemicals are used or isolated among 120 different species of ascidians! For example, you can look for an article you need by using a chemical name, a cas number of a chemical, an effect of a chemical as a search term. Some information on ACBD was acquired by personal communication by contacting ascidian researchers manually.

History of Ascidian Chemical Biology

650 articles have been published in the field of ascidian chemical biology.

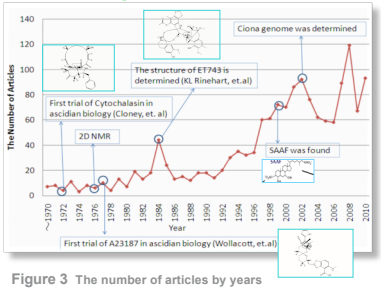

Figure 3 The number of articles by years

This figure shows how many articles are published in each year from 1964 to 2010. X axis shows year, and Y axis shows the number of records in ACBD (≒ the number of articles). Moreover, some important events in ascidian biology, such as complete of whole-genome sequencing of *Ciona intestinalis*, are indicated in this figure.

The number of articles in each category

Category I: adding chemicals to ascidians (9 subcategory)

Category II: isolated chemicals from ascidians

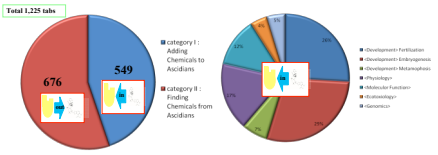

Figure 4 Number of articles by categories

The articles in ACBD are divided into 2 categories. One is 'Adding chemicals to ascidians'. We classified such kind of articles as Category I, which is the study that the chemicals are applied to ascidians to investigate some biological mechanisms of ascidians. For instance, metamorphosis, fertilization and embryogenesis etc. This category (Category I) is able to be classified into sub-categories depend on the purpose of study. Another category is Category II, 'Finding Chemicals from Ascidians'. This category describes studies that new chemicals are isolated or just found from ascidians.

In Category I, 'Adding chemicals to ascidians' is able to be classified into more detailed categories (subcategories). We made 7 different kinds of subcategories: (i) <Development>fertilization, egg maturation, or sperm activity; (ii) <Development>embryogenesis; (iii) <Development>metamorphosis; (iv) <PHYSIOLOGY>; (v) <MOLECULAR FUNCTION>; (vi) <ECOTOXIOLOGY> and (vii) <GENOMICS>. For example, "(i) <Development>fertilization, egg maturation, or sperm activity" includes the articles describing Ca2+ oscillation in egg maturation. (iv) <Physiology> includes the articles describing allorecognition. (vi) <ECOTOXIOLOGY> includes the articles describing the way to evaluate some marine paints' influence toward marine environment by using ascidians. From Fig. 3, we can say that approximately 70 % of articles are describing fertilization or embryogenesis.

Kinds of chemicals in each Category I and II

750 kinds of chemicals are referred in the articles.

Category of Chemicals	Kinds of Chmicals
NOT From Ascidians	351
From Ascidians	399
Total	750

Table 1 Total number of chemicals in Category I and II

Frequently used chemicals in Category I and II

Cytochalasin family is the most frequently applied chemical to ascidians. ET-743 is the most frequently referred chemical in Category II.

Table 2-1 Frequently used chemicals in Category I

Name of Chemicals	Number of Articles	PubMed (Ascidian+Chem name)	Effect
Cytochalasin Family	55	57	inhibit cellular processes such as cell division, and even cause cells to undergo apoptosis
A23187	12	19	a mobile ion-carrier that forms stable complexes with divalent cations (ions with a charge of +2)
U0126	11	8	MEK1 and MEK2 inhibitor
Puromycin	9	13	causes premature chain termination during translation taking place in the ribosome
Leupeptin	9	7	an inhibitor of calpain
Aphidicolin	8	9	a reversible inhibitor of eukaryotic nuclear DNA replication. It blocks the cell cycle at early S-phase
Actinomycin D	8	18	an investigative tool in cell biology to inhibit transcription
Nocodazole	5	9	interfering with the polymerization of microtubules

Table 2-2 Frequently used chemicals in Category II

Name of Chemicals	Number of Articles	PubMed (Ascidian+Chem name)	Effect
Ecteinascidin Family	37	22	anticancer drug
Eudistomin Family	27	10	strong antiviral activity against Herpes simplex virus and certain types of tumors
Didemnin Family	13	13	anticancer drug
Lepadiformine	12	13	antiarrythmic properties
SAAF	11	10	Sperm-activating, attracting factor
Lepadin	11	4	biological activities ranging from cytotoxicity, inhibitions of tyrosine kinase, anti-plasmodial and anti-trypanosomal properties as well as anti-malarial properties
Patellamide Family	8	12	anticancer drug

Table 2 Frequently used chemicals in ascidian biology

This tables show how many times each chemical was described in articles from 1964 to 2010, and the effect of each chemical. In detail, Table 2-1 describes chemicals applied to ascidians. From this table, we can find that the most frequently used chemical in Category I is Cytochalasin Families. These chemicals are usually used to stop cell division (cleavages). The second one is A23187 used as an ionophore. Table 2-2 describes the chemicals derived from ascidians. From this table, we can find that ET-743 is the most frequently described chemical in Category II. This chemical compound is isolated from the ascidian, *Ecteinascidia turbinate.* ET-743 acts as an anticancer drug. Some chemicals derived from ascidians which have medical effect to human.

Frequently used ascidians in Category I and II

Ciona intestinalis is the most frequently used ascidians both in category I and II.

Table 3-1 Frequently used ascidians in Category I

Species	Kinds of Chemicals ever added	Number of articles in Category I
Ciona intestinalis	84	162
Halocynthia roretzi	56	78
Phallusia mammillata	25	32
Botryllus schlosseri	16	27
Ciona savignyi	12	18

Table 3-2 Frequently used ascidians in Category II

Species	Kinds of Chemicals ever isolated	Number of articles in Category II
Ciona intestinalis	16	26
Lissoclinum cf. badium	15	15
Halocynthia roretzi	14	21
Lissoclinum patella	14	19
Didemnum psammatode	13	14

Table 3 Frequently used ascidians in category I and II

These tables show how many times each ascidian species was used in articles from 1964 to 2010, and the kids of chemicals ever applied to these species or ever isolated from these species.

In detail, table 3-1 describes species to which chemicals applied. From this table, we can find that the most frequently used species in Category I is *Ciona intestinalis*.

Table 3-2 describes the species from which chemicals were isolated. From this table, we can find that *Ciona intestinallis* is the most frequently used species also in Category II. Such chemicals isolated from *Ciona* are reported to have an anti-cancer effect.

Effects of Chemicals ever used in ascidians biology

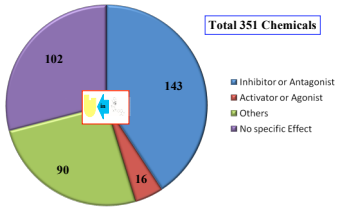

Figure 5 Effects of Chemicals in Category I

This figure shows effects of chemicals in Category I. The total number of chemicals in Category I is 351. Among them, the number of chemicals with no specific effects is 102. The examples of this kind of chemicals are cAMP, Mannose, and Glucose. The number of chemicals with effects as inhibitors is 143. For example, SB431542, Apaidicolin and Puromycin are included in this category. In contrast, the number of chemicals with effects as activators is only 16. Carbachol, Caffein, and Mastoparan are examples of this category.

Table 4 Kinds of chemicals ever applied to ascidians

Category of Inhibitor	Kinds of chemicals	Category of Inhibitors	Kinds of chemicals
Protein Kinase Inhibitor	3	Protein/RNA Transporter Inhibitor	1
G Protein_2nd Messanger Inhibitor	7	Ca Signal and Channel Inhibitor, Neural Transmission Inhibitor	24
Calmodulin Kinase Inhibitor	6	Caspase, Proteasome, Granzyme B, Secretase Inhibitor	4
Cyclic-dependent Kinase Inhibitor	4	Proteinase Inhibitor	18
MAPK Signal Inhibitor	6	Cox, Oxidant Stress, NO related Inhibitor	8
PTK inhibitor	5	Appotosis-indusing drug Inhibitors	4
Wnt Signal Inhibitor	2	Angiogenesis Inhibitor	3
PI3K-Akt Signal Inhibitor	2	Cell Cytoskelton, Cell Division Inhibitor - Actin Skeltal System-	6
Notch Signal Inhibitor	2	Cell Cytoskelton, Cell Division Inhibitor - Microtuble Skeltal System-	5
Protein Phosphatase Inhibitor	4	Telomerase Inhibitor	1
Cytokine Signal Inhibitor	1	Sugar Prosessing related inhibitors	3
Hormone Signal Inhibitor	4	Anticancer Drug	2
HDAC Inhibitor	0	DNA, RNA translation process related inhibitor	11
NF κB Inhibitor	1	Lipoxygenase and peroxidase	6

Table 4 Kinds of inhibitors ever applied to ascidians

This table shows what kind of inhibitors were used to ascidians by now. According to Figure 5, the total number of inhibitors ever used to ascidians is 143. Table 4 shows that among the 143 inhibitors, most frequently used inhibitor is "Ca Signal and Channel Inhibitor, Neural Transmission Inhibitor." The number of chemicals which belong to this category is 24. The second largest category is "Proteinase inhibitor." The total number of chemicals in this category is 18. Additionally, from table 4, we can say that various inhibitors were already used in ascidians biology.

The number of Ciona protein models that could be targets of chemicals

Based on the information from articles, we found that 199 *Ciona* genes could be targets of chemicals. Additionally, based on Blast search results, we found that further 1,777 *Ciona* protein models could be targets of chemicals (never applied to ascidians).

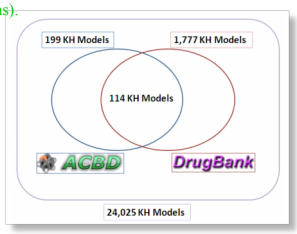

Figure 6 The number of *Ciona* protein models

This figure shows how many *Ciona* protein models could be targets of chemicals. Based on transcriptome analysis (Satou et a., 2008 *GenomeBiol*.), total *Ciona* transcript was deduced to be 24,025. Among them, based on the information from articles, we found that 199 proteins encoded by the 199 genes have ever targeted by chemicals. To know the number of potentially modifiable target proteins by currently available chemicals, we searched KH model which can be potentially a drug targets of chemicals recorded in DrugBank (Wishart DS et al., 2008). As a result, we can say that 1,862 KH *Ciona* models might be modifiable by using commercially available chemicals. As a conclusion, we found that around 7.8% of *Ciona* models have the possibility to be modified their functions by being applied chemicals.

Conclusion

Through the process of making our database, we could overview the history of chemicals in relation to the biology of ascidians since 1964. From this history, we can say that frequency of using chemical compounds in the field of ascidian biology are increasing in recent years, and almost 750 kinds of chemicals are used/isolated in 120 species of ascidians. More than 391 potentially valuable chemicals are isolated from ascidian (actually, ET-743 from ascidians are already used in our life as an anti-cancer drug). On the other hand, 351 kinds of chemicals have ever used for targeting or modifying specific protein function in ascidians. In addition, we found that, 1,862 kinds of *Ciona* proteins (7.8% of all *Ciona* models) might have the potential to be targeted by using commercially available chemical compounds. Thus ACBD is a good platform for the ascidian chemical genomics and drug screenings as well as ascidian chemical biology.

Future Work

ACBD will be linked to related-ascidian database like CIPRO and ANISEED.

Chemical inhibitors can be specifically knock-down a protein function. Thus, we are now developing a high-throughput whole-animal phenotype screening system using *Ciona.*

ACBD will be published in near future. Preliminary beta version of ACBD is already available and the URL is below.

http://chordate.bpni.bio.keio.ac.jp/acbd/top.html

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「化学発生生物学展開のためのホヤを用いた小分子化合物高速スクリーニング」