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Biocomplexity Faculty Search Committee,
c/o Prof. Rob de Ruyter van Steveninck
Biocomplexity Institute,
Indiana University,
Swain Hall West 117,
Bloomington, IN 47405-7105

Dear members of the search committee,

I am submitting my application materials to be considered for the advertised faculty position. I am trained as a biologist by Dr. Bruce Hammock at University of California, Davis and Dr. Peter Schultz at the Scripps Research Institute. They have both fueled my interest in applying genome-scale functional analysis and chemical genetic approach to biological and pharmacological research. During my graduate studies I cloned and characterized genes involved in cell death signaling pathways. My postdoctoral work here in the Schultz lab has focused on developing and applying new high-throughput technologies for functional genomics analysis in various systems, mammalian cell, mouse and yeast. I recently developed and applied array-format high-throughput transfection to identify novel regulators of tumor suppressor protein p53 in a cell-based assay. I also did a screen for novel G2/M checkpoint genes using a complete collection of yeast homozygous deletion strains and identified rts1 as a G2/M checkpoint gene in yeast. Currently I am using phenotype-driven forward genetic approach to identify cancer resistant mouse in large-scale mutation isolations by the mouse supermutagen N-ethyl-N-nitrosourea (ENU). I feel these combinations of genome-scale analysis and molecular characterization have prepared me well for a career in biological and pharmacological studies by functional genomics approaches.

The array-format cDNA and RNAi technology developed in the Schultz lab is quite powerful. My primary interest as an independent investigator is to develop new high-throughput technologies, apply these tools to discover novel drug targets and study the function of these target proteins. Specifically, I am interested in developing array-format RNAi technologies for phenotype-driven screens in the genetic model systems such as mouse, zebrafish and drosophila, etc.. In the application portion of my research, I am interested in using synthetic lethality to discover potential chemotherapy drug targets that together with mutations in tumor suppressor genes such as p53, ARF, etc. or oncogenes such as Ras, Myc, etc. will selectively kill cancer cells. These should be selective targets as normal cells with intact pathways will be unaffected. These targets will be investigated by array-format RNAi and chemical genetic approaches. This is a completely novel approach to chemotherapy drug discovery and is only made possible by recently developed technologies. I am enthusiastic to begin this work as it will lead to drug targets and chemotherapies that may be effective in the treatment of a broad spectrum of tumors. The small molecules found in these screens may also be more selective than conventional chemotherapy drugs and have fewer side effects. I am also thrilled about the prospect of teaching students in the area of functional genomics, biotechnology, pharmacology and molecular biology.

The multidisciplinary nature of research in your center would make an ideal setting in which to pursue my career goals. Likewise, I think that I would be a creative and energetic addition to the group.

Enclosed please find my curriculum *vitae* and a brief summary of my future research plans. I have asked Professor Schultz, Hammock and Reed to forward letters of recommendation to you directly on my behalf. Thank you very much for your consideration.

Sincerely,

Qihong Huang

Qihong Huang

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Identification of cancer drug targets by RNAi and chemical genetics

Cancer chemotherapy drugs have traditionally targeted the aberrant proliferation of tumor cells. The mechanism of action of these drugs include direct or indirect DNA damaging agents, mitotic arrest agents, purine and pyrimidine antimetabolites and steroid signaling inhibitors (1). Unfortunately these small molecules are not specific to tumor cells and cause severe side effects. Over the past decade, some correlations between special genetic lesions and cancer development have been identified and have prompted the development of selective small molecule and antibodies that target the causative proteins in cancers (2, 3, 4). However, these agents are only limited in the treatment of few types of cancer. In order to find targets for the next generation of cancer selective chemotherapy, it will be necessary to use a variety of recent developed new technologies such as RNAi and chemical genetics.

Synthetic lethality was first described in Drosophila when mutations at additional loci enhance or suppress the phenotype caused by a particular mutation (5). Two mutations are considered synthetic lethal if in combination they result in cell death, whereas either alone leads to a viable cell. One common feature of all cancers is the impairment of p53 pathway. P53 is a tumor suppressor protein which is mutated or deleted in almost 50% of all cancer. I propose to use synthetic lethality to discover potential drug targets that together with a p53 mutation will selectively kill cancer cells. These should be selective targets as normal cells with intact p53 will be unaffected. These targets will be investigated by array-format RNAi and chemical genetic approaches.

Isogenic mammalian cell lines which have the same genetic background except p53 status have been created (6, 7) and are quite useful for synthetic lethality screening. Array-format short-hairpin RNA (shRNA) or small molecules are a collection of RNAs or small molecules that are spotted in multi-well plates so that each well contains individual sequence-annotated shRNA or small molecule. These libraries provide a means to systemically identify drug targets and chemotherapy lead compounds in cell-based assays. It also provides a direct link between genotype and phenotype in these assays. These shRNAs or small molecules will be transfected or added to isogenic cells by a high-throughput method (8, Figure 1). Viability assays such as alamar blue assay can verify cell death in cancer cells. Agents that selectively cause cell death in p53-/- cells but not in p53+/+ cells will be selected and confirmed, and the mechanisms of synthetic lethality by these agents will be further characterized. Previously in the Schultz lab, I conducted a chemical cytotoxicity screen on a pair of isogenic human lymphocytes that are identical apart from p53 status and was able to identify several small molecules that selectively cause cell death in p53-/- cells but not in p53+/+ cells. Analogs of these small molecules and their linker derivatives will be attached to resin to make affinity matrices. Cell extract from this pair of cells will be incubated with these affinity supports and target protein will be purified by affinity chromatography. Elutions will be analyzed by SDS-PAGE gel and protein bands will be excised and sequenced. The proteins that bind to the small molecule represent potential drug targets and can be further confirmed by RNAi. Similarly, systemic RNAi cytotoxicity screen will be performed on isogenic p53

cell lines to identify selective targets that cause cell death in p53-/- cells but not p53+/+ cells. These selection cytotoxicity screens will reveal mutations that cause synthetic lethality when combined with the genetic lesion in p53 pathway. Information from the RNAi screen and chemical cytotoxicity screen can be used in conjunction with data derived from gene expression profiles of the same cell (Figure 1). Similar screens can also be applied to cancer cells harboring oncogenes that maintain malignancy such as Ras and Myc.

These screens will lead to drug targets and chemotherapies that may be effective in the treatment of a broad spectrum of tumors since p53 pathway impairment is a shared characteristic in most cancers. The small molecules found in this screen may also be more selective than conventional chemotherapy drugs and have fewer side effects.

This project is an extension of my skills learned in my postdoctoral and graduate studies. I did a high-throughput cellular screen using an array-format matrix containing approximately 20,000 cDNAs and 10,000 shRNAs in Schultz lab. That experience will be helpful for the systemic RNAi synthetic lethality screening. I also found several small molecules that selectively kill p53-/- cells whereas p53+/+ cells are not affected. These molecules can be used to find out the target proteins that together with p53 deletion will cause cell death. I believe I am well prepared to begin this project. The funding of this project will be sought from NIH, NSF and other private funding agencies.

The array-format high-throughput technology will be useful and available to all labs who are interested in phenotype-driven genetic screens. These new technologies developed in my program will complement other "omic" technologies on campus. The data collected from all the screens will be deposited in a central data base including microarray, IP-MS, metabolic analysis, etc. so that for any hits from the screen, researchers will be able to look up immediately its gene expression pattern in cell lines and tissues (genomic, transcriptomic), proteins it associate with (proteomic), which metabolic pathways it is involved (metabolomic) and functions in other screens. This sum of parts will speed up the annotation and characterization of gene function, discovery of potential therapies for human diseases and encourage collaborations between individual labs on campus.

References:

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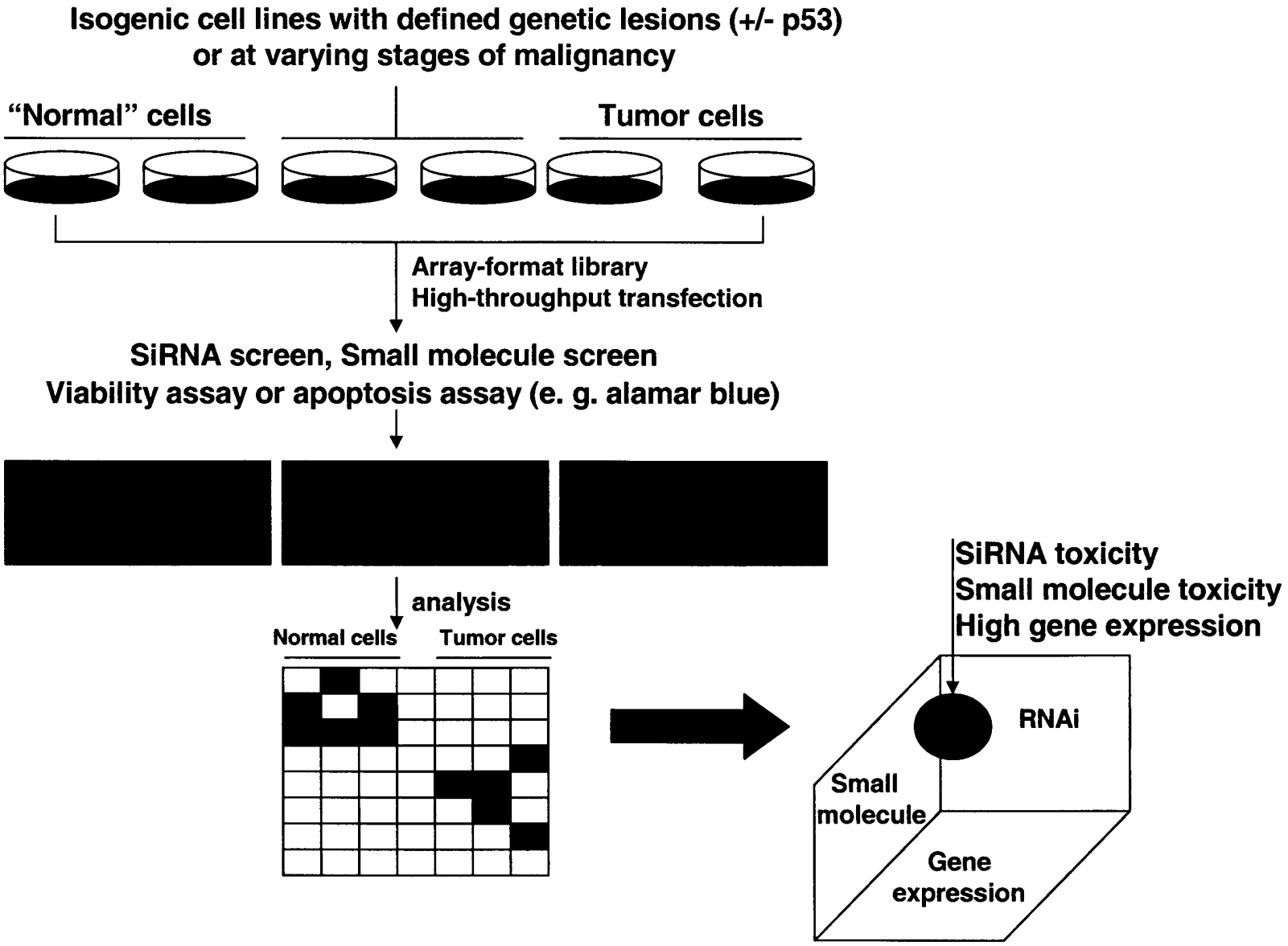
Figure 1


Isogenic cell lines with defined genetic lesions (+/- p53)
or at varying stages of malignancy
"Normal" cells
Tumor cells
Array-format library
High-throughput transfection
SiRNA screen, Small molecule screen
Viability assay or apoptosis assay (e. g. alamar blue)
analysis
Normal cells
Tumor cells
SiRNA toxicity
Small molecule toxicity
High gene expression
RNAi
Small molecule
Gene expression

Summary of Teaching Interests

Qihong Huang

Today the line between biology, chemistry and engineering are becoming less clear and require future scientists to be more interdisciplinary in nature. The development of new technologies and the completion of the genomes of various model organisms provide opportunities in interdisciplinary research using high-throughput methods. I am glad that my training has been focused on the development and application of high-throughput technologies in these researches. The prospect of sharing my knowledge with students as a teacher and research advisor is very exciting. I would be happy to teach introductory molecular biology, biochemistry, microbiology and upper level courses in pharmacology, chemical genetics and biotechnology. I would also be interested in developing an elective course on functional genomics. This course would first cover the principles of current high-throughput technologies such as RNAi, small molecule, cDNA, ENU mutant mice, etc.. It will then discuss the application of these technologies in biological, chemical research and drug discovery, taking specific research topic and covering the screen methods design, optimization, data analysis, and candidate confirmation and characterization.

Ideally, I think that both students and professors should learn during class and this is only facilitated in an environment that elicits student inquiry and participation. It is much more important to students to find the subject matter interesting than easy to digest, I think an effective methods for holding students' interest is to incorporate topics pertinent to their everyday lives into the curriculum. As just one example, students might be indifferent to a lecture on 'genetic engineering' in a typical molecular biology class. They may take note however, if it covered the creation of genetically modified corns, tomatoes and their effects on human health and environment because it is clear to see that these topics are also relevant outside of the classroom.