

CURRICULUM VITAE

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Education

Ph.D. (Specialty: **Genetics**) Sept. 1999---July 2002, Laboratory of Ophthalmology & Medical Genetics, Eye Research Institute, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, P. R. China.

Thesis supervisor: Prof. Qing-Jiong Zhang

Thesis topic: Primary Proteomic Study on the Retinas of Rds, Rd and Their Normal Control C3B mice

M.Sc. (Specialty: **Zoology**). Sept. 1994---July 1997, School of life science, Zhongshan University, Guangzhou, P.R. China.

Thesis supervisor: Prof. Hao-Ran Lin (Academician)

Thesis topic: Isolation and Purification of Immunoglobulin M (IgM) and Variation of Maternal IgM during the Development of Oocyte and Embryo in Crucian Carp (*Carassius auratus*)

B.Sc. (Specialty: **Zoology**). Sept. 1987---July 1991, Department of Biology, Zhongshan University, Guangzhou, P.R. China.

Work Experiences

2002/7---now, **Assistant professor**, Cancer Center, Sun Yat-sen University

1997/7---1999/9, **Assistant professor**, Pearl River Institute of Fisheries, Guangzhou.

1991/8---1994/7, **Teaching assistant**, Yulin High Training School, Shaanxi Province,

Special skill

Genetics: **DNA and RNA manipulation** (DNA and RNA extract, Southern blotting, Northern blotting, Cloning, Nested PCR etc.)

Cytobiology: **Protein** (protein purification, column chromatography, antibody preparation, cytochemistry, immunohistochemistry, RIA, microscopy/electron microscopy, Western blotting, 2D-electrophoresis, MS spectrometry, proteomic analysis, etc.)

Cell (cell culture etc.)

Animal model: **Fish, mouse and rat** manipulation.

Virus: **Epstein-Barr virus**

Computer: Familiar with the **Microsoft operating system** (MS-DOS6.22, Windows 3.2, Windows 95, Windows XP), **Microsoft Office XP**, lots of application systems and **biology software**, such as Primer5.0, Clustalx1.83, Vector NTI, DNASTAR, Phylip etc.

References, Transcripts and Writing Sample Available Upon Request

Current work

After my Ph.D. study, I got an assistant professor position in Cancer Center of Sun Yat-sen University. My major work was to study the relationship between the nasopharyngeal carcinoma (NPC) and the Epstein-Barr virus (EBV). Firstly, we established an EBV strain (Guangdong 1, GD1) from an Cantonese NPC patient and sequenced its whole genomic sequence, which published in our paper “Genomic Sequence Analysis of Epstein-Barr Virus Strain GD1 from a Nasopharyngeal Carcinoma Patient” *J Virol.*, 2005, 79(24). The results showed there were many sequence variations in GD1 compared to prototypical strain B95.8, including 43 deletion sites, 44 insertion sites, and 1,413 point mutations. The frequency of some of these GD1 mutations in Cantonese NPC patients showed they were highly prevalent. These results suggested that GD1 was highly representative of the EBV strains isolated from NPC patients in Guangdong, China, an area with the highest incidence of NPC in the world. This work provided us basic information of EBV in Cantonese. Meanwhile, we also wanted to genotype the NPC-related EBV by several genes. The results showed there was a special subtype EBV existing in NPC patients, which latently functional genes have some linked variants. This paper was in prepared. I'm also analyzing the oncogenesis activity of GD1, mechanism of genes of EBV in the course of NPC.

Summary of Ph.D. Research

Primary Proteomic Study on the Retinas of Rds, Rd and C3B Mice

Major: Genetics

PhD Candidate: Da-Jiang Li

Supervisor: Qing-Jiong Zhang, Prof.

Laboratory of Ophthalmology & Medical Genetics, Eye Research Institute,
Zhongshan Ophthalmic Center, Zhongshan University, Guangzhou, P. R. China

Retinitis pigmentosa (RP) is characterized by constriction of the visual fields, night blindness, and fundus changes, including 'bone corpuscle' lumps of pigment. RP unassociated with other abnormalities is inherited most frequently (84%) as an autosomal recessive, next as an autosomal dominant (10%), and least frequently (6%) as an X-linked recessive in the white U.S. population. The overall frequency was estimated at about 1 in 3,700, whereas the incidence of the recessive type, with at least two genocopies, was estimated to be about 1 in 4,450. No evidence of ethnic heterogeneity was found.

Retinal degeneration (rd), and retinal degeneration, slow (rds) is well-accepted animal model of RP. In order to identify proteins involved in the course of RP, we analyze the proteomic differential expression in the retinas of rd, rds mice as compared with that of normal C3B mice.

Purpose: We analyzed the proteomic differential expression in the retina of rds, rd mice as compared with that of normal C3B mice in order to find some proteins involved in the course of RP. After that the functions of the proteins were studied.

Materials and Methods: Retinal proteins were collected from rd, rds mice and C3B mice respectively at P7 (postnatal day 7), P12, P21 and P37. The proteins were separated using 2-DE. Several differentially expressed protein spots were excised from the gels and in-gel digested by trypsin, and then subjected to peptide analysis by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) or liquid chromatography-mass spectrometry (LC-MS). The corresponding mRNA of those differentially expressed proteins was analyzed by using RT-PCR.

Results: A lot of differentially expressed proteins in the retina were found between rds, rd and C3B mice in 2-DE. Four protein spots were identified as PSD95, UP2, GRB14 and HPCAL1/NCALD. In the 2-DE gel, protein PSD95 and UP2 in rds expressed lower than that of C3B at P37, HPCAL1/NCALD in rd expressed higher than that of C3B mice at P37, and GRB14 in rds expressed higher than that of C3B at P21, which means they may involved in the course of RP. All the 4 proteins' genes showed transcriptional changes through RT-PCR analysis too. PSD95 mRNA in rds was higher than that of C3B at P7, but was lower at P12, P21 and P37. There was a lowest part of PSD95 transcription in rds at P12 when its retina developed quickly; PSD95 mRNA in rd was

higher than that of C3B at all four ages. The significant changes of PSD95 in rds and rd mice determined that PSD95 involved in the course of RP greatly, and worked in different ways in rds and rd mice. UP2 mRNA in rds and rd expressed closely to that of C3B at P7, and much higher than that of C3B at P12, P21, but was lower than that of C3B at P37. This means that UP2 involved in the course of rds and rd in the same way. We have identified one protein may be HPCAL1 or NCALD too. HPCAL1 mRNA in rd was higher than that of C3B at all four ages; but it was lower in rds than that of C3B at P7, little higher than that of C3B at P12, P21 and much higher at P37, so HPCAL1 influenced on rd more than on rds. NCALD mRNA in rds expressed lower than that of C3B at P7, P12, P37, and little higher than C3B at P21, while in rd it was higher at P12, P21 and lower at P7, P37 than that of C3B, that means NCALD may influenced on rds more than on rd. GRB14 mRNA in rds and rd was almost identical to that of C3B at all four stages except at P37 in rds was lower than C3B, so it has little influence on the development of RP. PSD95, UP2, HPCAL1 and GRB14 mRNA changes coincided with its 2-DE result, so they should be the protein spots cut from the gel.

Conclusion: There are lots of proteomic differentiation in the retina of rds, rd and C3B mice during their development. PSD95, UP2, GRB14, HPCAL1 (or NCALD) were found to be expressed differentially, which implies that they may involve in the course of RP.

An abstract summarizing this research has been accepted for the 2002 annual meeting of *Association for Research in Vision and Ophthalmology*, which has been held in Florida, USA, from May 5 to 10, 2002.

LIST OF PUBLICATIONS

Note: Paper [1] to [4] have been published recently, which were parts of my works on Oncology and Virology.

[1] Zeng MS, **Li DJ**, Liu QL, Song LB, Li MZ, Zhang RH, Yu XJ, Wang HM, Ernberg I, Zeng YX. Genomic Sequence Analysis of Epstein-Barr Virus Strain GD1 from a Nasopharyngeal Carcinoma Patient. *J Virol.*, 2005; 79(24): 15323-15330. (English)

[2] Mai SJ, Zhang XS, **Li DJ**, Shen GP, Jiang JH, Zhang RH, Yu XJ, Chen SP, and Zeng YX. 2004. The character of EBNA1 gene variation in Cantonese and its association with nasopharyngeal carcinoma. *Chinese Science Bulletin* 49:1~4. (Chinese and English)

[3] Li JT, Liu W, Kuang ZH, Chen HK, **Li DJ**, Feng QS, Liu QC, Hu B. Amplification of RIT1 in hepatocellular carcinoma and its clinical significance. *Ai Zheng*, 2003; 22(7): 695-9. (Chinese)

Note: Paper [4] to [6] be about my works on molecular biology and proteomic.

[4] **Li DJ**, Zhang QJ. Transcriptional changes of α -crystallins gene during the development of retinal degeneration in rd, rds and C3H mouse. *Eye Science*, 2002; 18: 115~118. (Chinese)

[5] **Li DJ**, Zhong MC, Lin HR. Localization of Maternal Immunoglobulin in the Egg and Embryo of Crucion Carp (*Carassius auratus*). *Acta Scientiarum Naturalium Universitatis Sunyatseni*, 2001; 40(2): 82~85. (Chinese)

[6] **Li DJ**, Zhang QJ. Primary studies on retinal proteome of rds and C3B mice by two-dimensional gel electrophoresis. *Recent Advances in Ophthalmology*, 2001; 21(3): 153~156. (Chinese)

Note: Paper [7] to [13] be about my works on Zoology.

[7] Hu YC, Chen KC, Li HS, Deng GC, Wu GM, **Li DJ**. Feeding habits of *Mystus guttatus* in the Pearl River. *Jounral of Fishery Science of China*, 2003; 27(4): 301~306. (Chinese) [8] Chen KC, Wu GM, Li HS, **Li DJ** et al. Study on the age and

growth of *Mystus guttatus*. *Journal of Fishery Science of China*, 1999; 6(4): 62~66. (Chinese)

[9] Wu GM, Chen KC, Luo JR, Lin GG, **Li DJ** et al. The effects of salinity on the embryonic development of the *Clarias fuscus*, *Clarias lazera* and there hybrid F₁. *Journal of Fishery Science of China*, 1998; 5(3): 43~46. (Chinese)

[10] Chen KC, Wu GM, Luo JR, Lin GG, **Li DJ** et al. Effects of temperature on the embryonic development of the whitespotted catfish (*Clarias fuscus*), leather catfish (*Clarias lazera*) and there hybrid F₁. *Supplement to the Journal of Sun Yat-sen University*, 1998; 6: 53~57. (Chinese)

[11] Wu GM, Chen KC, Li HS, **Li DJ** et al. Present condition and future of channal catfish fishery in China. *Supplement to the Journal of Sun Yat-sen University*, 1998; 4: 75~79. (Chinese)

[12] Li HS, Wu GM, Fan Y, Chen KC, **Li DJ** et al. Primary research on reproduction biology of black crappie. *Supplement to the Journal of Sun Yat-sen University*, 1998; 4: 80~83. (Chinese)

[13] Wu GM, Chen KC, Luo JR, Lin GG, **Li DJ** et al. Oxygen consumption rate of whitespotted freshwater catfish, leather catfish and their hybrid F₁. *Journal of Fishery Science of China*, 1998; 5(4): 118~121. (Chinese)

Manuscripts prepared:

- [1] **Li Da-Jiang** et al. Effect of five heavy metal ions on hypophyseal -gonadal axis of common carp (*Cyprinus carpio*)
- [2] **Li Da-Jiang** et al. Proteomic study on the retinas of rd, rds and C3B mice as well as reconfirming of the differentially expressed proteins by using RT-PCT
- [3] **Li Da-Jiang** et al. NPC-related EB Virus subtypes in Guangdong of China.

Abstracts of papers published

[1] Zeng MS, *Li DJ*, Liu QL, Song LB, Li MZ, Zhang RH, Yu XJ, Wang HM, Ernberg I, Zeng YX. Genomic Sequence Analysis of Epstein-Barr Virus Strain GD1 from a Nasopharyngeal Carcinoma Patient. *J Virol.* 2005; 79(24):15323-15330.

Abstract: To date, the only entire Epstein-Barr virus (EBV) genomic sequence available in the database is the prototype B95.8, which was derived from an individual with infectious mononucleosis. A causative link between EBV and nasopharyngeal carcinoma (NPC), a disease with a distinctly high incidence in southern China, has been widely investigated. However, no full-length analysis of any substrain of EBV from this area has been reported. In this study, we analyzed the entire genomic sequence of an EBV strain from a patient with NPC in Guangdong, China. This EBV strain was termed GD1 (Guangdong strain 1), and the full-length sequence of GD1 was submitted to the GenBank database. The assigned accession number is AY961628. The entire GD1 sequence is 171,656 bp in length, with 59.5% G+C content and 40.5% A+T content. We detected many sequence variations in GD1 compared to prototypical strain B95.8, including 43 deletion sites, 44 insertion sites, and 1,413 point mutations. Furthermore, we evaluated the frequency of some of these GD1 mutations in Cantonese NPC patients and found them to be highly prevalent. These findings suggest that GD1 is highly representative of the EBV strains isolated from NPC patients in Guangdong, China, an area with the highest incidence of NPC in the world. Furthermore, these findings provide the second full-length sequence analysis of any EBV strain as well as the first full-length sequence analysis of an NPC-derived EBV strain.

[2] Mai, SJ, Zhang XS, *Li DJ*, Shen GP, Jiang JH, Zhang RH, Yu XJ, Chen SP, and Zeng YX. 2004. The character of EBNA1 gene variation in Cantonese and its association with nasopharyngeal carcinoma. *Chinese Science Bulletin* 49:1~4. [Brief communications.]

Epstein-Barr virus (EBV) is closely associated with nasopharyngeal carcinoma (NPC), which occurs with highest incidence in Guangdong. However, EBV is ubiquitous and remains a life-long asymptomatic infection in the peripheral B lymphocytes of he

healthy carriers; thus it is supposed that there is a substrain of EBV with carcinogenesis potential in NPC high incidence area....

[3] Li JT, Liu W, Kuang ZH, Chen HK, *Li DJ*, Feng QS, Liu QC, Hu B. Amplification of RIT1 in hepatocellular carcinoma and its clinical significance. *Ai Zheng*. 2003; 22(7):695-9.

Abstract: BACKGROUND & OBJECTIVE: Previous study has demonstrated that high frequent gain of 1q was detected in hepatocellular carcinoma (HCC), 1q21-22 was identified as the minimum overlapping amplified region and might contain the candidate oncogenes involved in HCC. RIT1 gene is located in 1q21.3 region and is a member of Ras subfamily. RIT1 protein is similar to Ras protein in molecular structure and functions. It was speculated that RIT1 gene might be a candidate oncogene in HCC. So, the amplification of RIT1 gene was examined in HCC and was linked with the clinical indicators in this study to explore the possible functions of RIT1 gene in HCC development and progression. METHODS: The fluorescence quantitative polymerase chain reaction (FQ-PCR) method was established successfully. The number of RIT1 gene DNA copies was examined in the tumor tissues and its paratumor tissues from 43 patients with HCC by PE ABI 7000 Sequence Detector. The ratio of the number of RIT1 gene DNA copies between the tumor tissue and its paratumor tissue represented the extent of amplification of RIT1 gene DNA. RESULTS: RIT1 gene DNA was amplified in 11 cases (25.6%) among 43 patients. The mean survival time (15 months) of the RIT1 gene-amplification group is significantly shorter than that (34 months) of the non-amplification group ($P = 0.0009$); furthermore, the pathological grade and the extent of liver cirrhosis were significantly different between the RIT1 gene-amplification group and the non-amplification group ($P < 0.01$). CONCLUSION: The amplification of RIT1 gene might be one of the activation ways in HCC and might play an important role in HCC development and progression.

[4] *Li DJ*, Zhang QJ. Transcriptional changes of α -crystallins gene during the development of retinal degeneration in rd, rds and C3H mouse. *Eye Science*, 2002; 18: 115~118.

Abstract: Purpose To determine the relationship between retinal α A—crystallins and the development of retinal degeneration in rd, rds and C3H mouse. Methods Total retinal mRNA was prepared from the retina of rd, rds, C3H and control C3B mice during the progress of retinal degeneration (post-natal 3, 4, 5, 6, 8 weeks). The retinal expression of α A—crystallins was determined by semi-quantitative RT-PCR analysis. Result Expression of retinal α A—crystallins is rather stable in rd, rds and C3H mice during the progress of their retinal degeneration, which is similar to that in normal C3B mice but different to that in RCS rats. Conclusion Retinal α A—crystallins may not involved in advancing the retinal degeneration.

[5] *Li DJ*, Zhong MC, Lin HR. Localization of Maternal Immunoglobulin in the Egg and Embryo of Crucion Carp (*Carassius auratus*). *Acta Scientiarum Naturalium Universitatis Sunyatseni*, 2001; 40(2): 82~85.

Abstract: Immunoglobulin (Ig) were precipitated and purified from crucian carp's sera. After immunized with pure Ig of crucian carp in FCA and FIA, we obtained special antisera from rabbit. Immunohistochemical studies showed maternal Ig began to appear in the early IV phase egg. During the development of egg, Ig dispersed throughout the yolk of the egg and decreased from the membrane to the center, and more Ig localized inside the internal membrane than at other sites. Maternal Ig exist in the internal membrane, embryo body and yolk during embryonic development until heart beating stage and disappeared completely at the stage of caudal fin forming, Ig in embryo and yolk disappeared first, and then disappeared inside the internal membrane of egg. During the embryonic development, Ig also dispersed throughout the yolk of the embryo, and more Ig localized inside the internal membrane than at other sites; there was no phenomena of Ig concentrating in the embryo or yolk.

[6] *Li DJ*, Zhang QJ. Primary studies on retinal proteome of rds and C3B mice by two-dimensional gel electrophoresis. *Recent Advances in Ophthalmology*, 2001; 21(3): 153~156.

Abstract: Objective To Apply two-dimensional (2-D) gel electrophoresis to resolve specially expressed proteins related to retinitis pigmentosa (RP) in the retina of rds

mice. Methods Proteins, prepared from the retinas of rds mice and normal C3B mice at different ages, were separated by using two-dimensional electrophoresis and then analyzed by 2-DE imaging analyzer. Results 2D gel electrophoresis was established for the retinal proteome analysis. Retinal neuronal tissue was lysed by using chemical lysis solution and ultrasonic. Using carrier ampholyte to set up pH gradient as first dimension and casting vertical 12% SDS-acrylamide-bis slab as second dimension, the major retinal proteins showed maps of proteome on 2-D gels clearly. Retinal proteome of rds and C3B mice at 37d has different expressive patterns. Some proteins only expressed in the retina of rds mice while another only in the retina of C3B mice and others had different level between the two kinds of mice. Conclusion 2-D gel electrophoresis is effective to separate specially expressed retinal proteins. The expressed proteins in the rds retina are different in quality and quantity from that in the normal C3B mice.

[7] Hu YC, Chen KC, Li HS, Deng GC, Wu GM, **Li DJ**. Feeding habits of *Mystus guttatus* in the Pearl River. *Journal of Fishery Science of China*. 2003; 27(4): 301~306.

Abstract: This paper reports the feeding habits of *Mystus guttatus* in the Pearl River with natural resources investigation. The reports include feed fullness, feed composition, frequency of feed occurrence and variation of feed composition in different seasons was also studied. The investigation result shows that the feed of *Mystus guttatus* in the Pearl River are crustaceans, insect, annelida , fish and pieces of plant . Among them, the crustaceans and insects are the main feed; the emergence frequency is 78.6% and 45.8%, respectively. In different seasons, the variety of feed composition is markedly different, and no pause of feeding. The feed of *Mystus guttatus* changed with the different body length of the fish. The first feed change period was at about 170mm body length and the second at about 240mm body length. It is getting simpler as *Mystus guttatus* grows up.

[8] Chen KC, Wu GM, Li HS, **Li DJ** et al. Study on the age and growth of *Mystus guttatus*. *Journal of Fishery Science of China*, 1999; 6(4): 62~66.

Abstract: Some biological characteristics of spotted long barbell catfish *Mystus guttatus* were studied, including the age features of opercule and pectoral fin, the age composition of the catch from Pearl River and its branches. And the relationships of body length with opercule radius and with body weight were analyzed, as well as the growth ring formed during December to next February. The results show that the optimum material for age determination is opercule; the length –opercule radius relationship can be expressed as $L=34.76R+0.4533$. The length weight relationship equation is $W=0.032L^{3.1872}$. The growth of spotted long barbell catfish coped with Von Bertalanffy formula. The growth equations of body length and weight are respectively $L_t=156.75[1-e^{-0.089(t-0.0168)}]$ and $W_t=31193.17[1-e^{-0.089(t-0.0168)}]^{3.1872}$. The reasons for growth ring forming and growing characteristics were discussed. Meanwhile, a suggestion is put forward that for the spotted long barbell catfish growing in natural waters, the fishing should not be carried out until they grow to 6 years old and 3kg of body weight, and for those cultured by individuals, the fishing can be considered after the body weight reaches 2.5kg.

[9] Wu GM, Chen KC, Luo JR, Lin GG, **Li DJ** et al. The effects of salinity on the embryonic development of the *Clarias fuscus*, *Clarias lazera* and there hybrid F₁. *Journal of Fishery Science of China*, 1998; 5(3): 43~46.

Abstract: The effects of salinity on embryonic development of *Clarias fuscus* (F), *Clarias lazera* (L) and their hybrid F₁ (P) are that both the survival rate at some stages and hatch rate of them decrease as salinity increase. The suitable salinity is 5, the highest tolerance for salt concentrations while catfish can hatch is 10 for F, 11 for L and P. Salinity has little effect on the rate of their embryonic development. After all the rate will be decreased.

[10] Chen KC, Wu GM, Luo JR, Lin GG, **Li DJ** et al. Effects of temperature on the embryonic development of the whitespotted catfish (*Clarias fuscus*), leather catfish (*Clarias lazera*) and there hybrid F₁. *Supplement to the Journal of Sun Yat-sen University*, 1998; 6: 53~57.

Abstract: The results of our experiments show that water temperature is closely related to embryonic development of whitespotted catfish (*Clarias fuscus*), leather

catfish (*Clarias lazera*) and their hybrid F₁-pearl catfish. The optimal water temperature respectively ranges between 30~35°C for whitespotted catfish, 26~32°C for leather catfish and 24~34°C for pearl catfish, their maximum being 35°C and minimum 18°C. The hatching times of the three kinds of catfish in relation to water temperature show descent lines that equations are $T_F=401740^{-2.1765}$ for whitespotted catfish, $T_L=417760^{-2.2714}$ for leather catfish, and $T_P=416830^{-2.1946}$ for pearl catfish.

[11] Wu GM, Chen KC, Li HS, *Li DJ* et al. Present condition and future of channal catfish fishery in China. *Supplement to the Journal of Sun Yat-sen University*, 1998; 4: 75~79.

Abstract: This paper summarized the introducing history, current fishery condition and future of *Ictalurus punctatus*, emphasizing on analyzing several emergency problem in it.

[12] Li HS, Wu GM, Fan Y, Chen KC, *Li DJ* et al. Primary research on reproduction biology of black crappie. *Supplement to the Journal of Sun Yat-sen University*, 1998; 4: 80~83.

Abstract: This paper shows our results of primary research reproduction of black crappie *Pomoxis nigromaculatus*. Under nature condition in pond, when water temperature rise up to 16°C, black crappie begin to build up its nest and spawn. The nest is smooth and under water 15~40cm, round the root of grass near the bank, and the region of nest is about 20cm×30cm. Every nest has thousands of roe. The male fish guards its nest after the female spawn. Black crappie also spawns in artificial nest (20cm×40cm). In cement pool, it can spawn in artificial nest after injection of HCG, but the male don't guard the nest. The roe of black crappie is small, and its average diameter is 0.88mm. The egg has drop oil and egg interval is small. When water temperature is 19°C, the fertilized egg hatch after 48 hour. Under observing, the little fish swim out of its nest in 6th day after hatching and its yolk sac and oil drop disappeared in 8th day. The fry start to eat in 10th day. The initial food is rotifer.

[13] Wu GM, Chen KC, Luo JR, Lin GG, *Li DJ* et al. Oxygen consumption rate of whitespotted freshwater catfish, leather catfish and their hybrid F1. *Journal of Fishery Science of China* 1998; 5(4): 118~121. [Brief communications]