University of Cincinnati - College of Medicine
2013-2014 Elective Syllabi

OPHTHALMOLOGY

12 01 36

TITLE OPHTHALMIC RESEARCH

The student will participate in on-going research projects in ophthalmic laboratories. In consultation with the elective director, the student will develop a research project in the area of ocular molecular genetics using transgenic mice models to investigate eye development and diseases, therapeutic strategies in treating congenital and acquired eye diseases using techniques of gene therapy and stem cell transplantation.

DIRECTOR Winston . Kao, PHD
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Building: MSB Room: 5007

ADMINISTRATIVE SUPPORT PERSON Michele Wyan EMAIL: michele.wyan@uc.edu PHONE: 558-0815
BUILDING: MSB ROOM: 5110

INSTRUCTOR Chia-Yang Liu PhD
Mindy Call
Winston Kao PHD
Hongshan Liu MD, PHD

SITE Care Building

REPORT 1ST DAY CARE/Crawley 5860 8:30 a.m.

LENGTH 8 to 12 wks
CREDITS 16 to 24
MAX. ENROLL 2 FT/PT:Full-Time hours

ROTATION				MIN	MAX
1	7/1/2013	to	7/26/2013		2
2	7/31/2013	to	8/27/2013		2
3	9/3/2013	to	9/27/2013		2
4	10/1/2013	to	10/28/2013		2
5	10/30/2013	to	11/26/2013		2
6	12/2/2013	to	12/27/2013		2
7	1/2/2014	to	1/29/2014		2
8	1/31/2014	to	2/27/2014		2
9	3/3/2014	to	3/28/2014		2
10	4/1/2014	to	4/25/2014		2
11	4/29/2014	to	5/23/2014		2
12	5/26/2014	to	6/30/2014		0

WORKING HOURS 8:30 - 5:00 p.m.

INSTRUCTION

TYPE/FORMAT

40 hrs	Basic science research (one-to-one instruction)
2 hrs	Library time/independent study
3 hrs	Lectures, conferences, seminars, grand rounds, etc.
45 hrs total	

OPHTHALMOLOGY

RESPONSIBILITIES
Performance of procedural techniques
Attendance of conferences, grand rounds, research seminars, lectures
Reading of literature and research
Required topic presentations; one
Structured journal club/critical reading of literature presentation; two (2) structured

PREREQUISITES
Meet with Dr. Kao prior to enrollment

ORIENTATION
Winston W.-Y. Kao

TEACHING
50% Research faculty
50% elective director

FEEDBACK
Elective director

FINAL GRADE
10% Attitude, e.g., professionalism, motivation etc
10% Journal club, critical review of literature
20% Topic presentation
60% Research experiments
GRADE ASSIGNED BY: Elective director

OBJECTIVES

Knowledge/Skills

1. Learn basic laboratory skills, e.g., column chromatography, tissue culture, cloning, gene therapy strategy.
2. Acquire proficiency in methods to study ocular disorders, e.g., wound-healing in mouse corneas, use of monoclinal antibodies to localize components of extracellular matrix molecular biology, signaling transduction.
3. Regulation of gene expression during Embryonic Development using transgenic and knockout mice.
4. Surgery skills
5. Produce data which will be used for publication by combining the results obtained by other investigators in the laboratories.

Procedures

1. Perform basic laboratory techniques: pipetting, measure pH
2. Prepare solutions
3. Demonstrate simple surgical skill on ocular tissues
4. Molecular cloning
5. Perform immunohistochemistry

Attitudes (include professional attitudes/behaviors)

Not applicable

Main Course Topics (Provide list of key words for major concepts covered)

Molecular biology
Genetics
Protein chemistry
Wound healing
Ocular pathology
Development

Reading and/or Texts

Readings will be assigned according to the research project.

Other Resources - Computer resources

10 P.C.s are available for research

OPHTHALMOLOGY

Other Resources - Audiovisuals

Not applicable

Other Resources - Other (include learning stations, X-ray and image files, viewboxes, EKG reading

Laboratory space available

SAMPLE WEEK

Monday

8:00 am	8:30 am	Reading
8:30 am	9:00 am	Design Experiment
9:00 am	4:00 pm	Perform Experiment
4:00 pm	6:00 pm	Analyze Exp. Results

Tuesday

8:00 am	8:30 am	Reading
8:30 am	9:00 am	Design Experiment
9:00 am	4:00 pm	Perform Experiment
4:00 pm	6:00 pm	Analyze Exp. Results

Wednesday

8:00 am	8:30 am	Reading
8:30 am	9:00 am	Design Experiment
9:00 am	3:00 pm	Perform Experiment
3:00 pm	4:00 pm	Journal Club
4:00 pm	6:00 pm	Analyze Exp. Results

Thursday

8:00 am	8:30 am	Reading
8:30 am	9:00 am	Design Experiment
9:00 am	4:00 pm	Perform Experiment
4:00 pm	6:00 pm	Analyze Exp. Results

Friday

8:00 am	8:30 am	Reading
8:30 am	9:00 am	Design Experiment
9:00 am	4:00 pm	Perform Experiment
4:00 pm	6:00 pm	Analyze Exp. Results

OPHTHALMOLOGY

Schedule Note

OPHTHALMOLOGY

RESEARCH INTERESTS UNDERWAY IN THE OPHTHALMIC RESEARCH LABORATORIES

Roles of Mesenchyme-Epithelium Interactions on Morphogenesis during Eye Development, And Homeostasis And Wound Healing in Adult Corneas

The central hypothesis of our ongoing research is that the mesenchyme-epithelium interactions via signal transduction of growth factors and their receptors play pivotal roles in eye morphogenesis during development and in ocular homeostasis and wound healing in adults. Morphogenesis of cornea, conjunctiva and eyelids during vertebrate eye development involves the migration and differentiation of mesenchymal cells of neural crest origin, and the differentiation of cells of the surface ectoderm. The bi-directional mesenchyme-epithelium interactions via growth factors are essential for morphogenesis during development and homeostasis in adults. Growth factors (e.g., TGF-bs and FGF-7) play pivotal roles in modulating functions of mesenchymal cells of neural crest origin and differentiation of ectoderm cells during ocular morphogenesis. To examine this hypothesis, we have created mouse lines that over express transgene and ablate genes of interest in a cornea-specific manner with techniques of transgenesis and gene targeting.

Conventional gene ablation techniques have proven useful for elucidating gene functions. However, gene ablation often results in premature death of experimental animals and is therefore of limited value for the study of gene functions in adult animals. This difficulty can be overcome by using conditional gene ablation via gene targeting technique (Cre-Lox-P system). Cre is a phage recombinase that specifically deletes any DNA sequence between two Lox-P elements. The Lox-P elements can be engineered into mouse genome via gene targeting techniques, but the modified gene remains fully functional until its excision by Cre driven by cell type-specific promoter of transgenic mice.

We have isolated and characterized two cornea-specific genes. Keratin 12 (Krt12) and Keratocan (Kera) are expressed by corneal epithelium and keratocytes, respectively. Using knock-in strategy of gene targeting, we have prepared mouse lines that over express Cre recombinase in corneal epithelium of *Krt12Cre/+* mice. We have also obtained *Kera-Cre* transgenic mice that express Cre by corneal keratocytes via transgenesis. By crossing the cornea-specific Cre-transgenic mice to Lox-P mice, the modified gene will be deleted by the Cre recombinase activities, which will result in the ablation of the modified gene in the offspring. Thus, the offspring derived from such crossing will miss a functional gene in a cornea-specific manner. Many Lox-P mouse lines have been prepared by other investigators and can be used to breed our Cre mouse lines to generate mouse lines in which the Lox-P modified gene can be specifically ablated in keratocytes and corneal epithelial cells. Using these strategies, we are currently examining the roles of TGF-b and AP1 transcription factors on corneal morphogenesis during embryonic development and homeostasis in adult. For example, the ablation of TGF-b type II (*TbRII*) in corneal epithelium did not perturb corneal epithelial differentiation, but impaired the healing of epithelium debridement.

Using similar knock-in strategy, we have also created a *Krt12rtTA/+* mouse line that expresses reverse tetracycline transactivator (rtTA) by corneal epithelium. Cross breed of *Krt12rtTA/+* with tet-O-reporter mice will produce bitransgenic mice carrying both rtTA and tet-O-reporter genes in which the expression of reporter gene can be induced by feeding experimental mice doxycycline (so called tet-on system). We use this strategy to over express FGF7 (keratocyte growth factor, KGF) in corneal epithelium of the bitransgenic mice. Our preliminary results indicate excess FGF7 leads to corneal epithelium hyperplasia and corneal vascularization.

These *Krt12Cre/+*, *Krt12rtTA/+* and *Kera-Cre* mice will enable us and many other investigators in the field of corneal cell biology to study the effects of loss and/or gain of function of genes of interest on corneal morphogenesis during development

and homeostasis in adult. Studies using these mouse lines will bring the corneal cell biology forward and knowledge learned from the studies will have significant impacts on designing treatment regimens of corneal diseases.

Extracellular Matrix Biology

Proteoglycans are well known to play a critical role in corneal strength and transparency. Lumican, the most abundant one of stromal proteoglycans, belongs to the family of small leucine-rich proteoglycans (SLRP). Like other SLRP proteins, lumican serves as a regulator of collagen fibrillogenesis. Additionally, a growing number of cellular functions have been identified for lumican including mediation of cell migration during corneal wound healing, metastasis of tumor cells, induction of epithelial-mesenchyme transition (EMT) in injured lens, and alteration of keratocan gene (*Kera*) expression. It is hypothesized that via interaction with cell surface receptor(s) lumican exerts its diverse functions on cellular activity for the maintenance of tissue homeostasis. Attempts are being made to continue our ongoing characterization of structure/function relationships of lumican in respect to collagen fibrillogenesis, *Kera* expression, cell migration and gene expression in injured cornea, formation of PMN (polymorphonuclear neutrophils) during myelopoiesis, and isolation and characterization of Lum receptor. The domains of lumican identified to be active in these diverse functions can then be tested for potential use in treating diseases involving lumican.

Corneal transparency requires a high level of organization of stromal collagen fibrils. Keratan sulfate proteoglycans (lumican, keratocan and mimecan) are essential components in fibril organization, however, individual KSPGs have distinct roles in maintaining corneal transparency as evidenced by cloudy thin corneas of *Lum*-/- and transparent thin corneas of *Kera*-/- mice. We hypothesize the molecular mechanism regulating collagen fibrillogenesis by lumican but not by keratocan protein arises from different amino acid sequence of specific protein domains in these closely related proteins. The role of N- and C-terminal lumican domains on collagen fibrillogenesis will be determined by using minigenes carrying site-specific mutations, e.g., substitution of tyrosine by phenylalanine, cysteine by serine. Our studies indicated that lumican can regulate *Kera* expression *in vivo* and *in vitro*. To determine the domains of lumican which serve as the regulator of this process, minigenes expressing hybrid fusion proteins containing different domains of lumican and keratocan and also minigenes expressing truncated lumican molecules will be injected to the stroma of *Lum*-/- mice. After intrastromal injection, *Kera* mRNA and protein expression will be determined by western blot analysis, and northern hybridization using antibodies and 32P-labeled probes recognizing N- or C-terminal KERA domains, respectively. These results will allow design of experiments which fully elucidate the lumican domains involved in other cellular activities, e.g., gene expression, cell migration, tumorigenesis, etc.

In vitro, knockdown of lumican expression using siRNA caused concomitant down-regulation of both *Kera* and *Aldh* (aldehyde dehydrogenase) in primary cultured bovine keratocytes. Microarray analysis revealed that *Lum*-/- PMN exhibited down regulated expression of *Gpr33* (G-protein coupled receptor). These observations support the hypothesis that via interaction with cell surface receptor(s) lumican exerts its diverse functions on cellular activity for the maintenance of tissue homeostasis. To examine this hypothesis, microarray analysis will be performed to examine possible alteration of gene expression in unwounded and wounded corneas, the role of down regulated *Gpr33* on PMN chemotactic activity and formation of PMN during myelopoiesis in the absence of Lum.

To further examine whether lumican has a role in PMN formation, bone marrow cells isolated for *Lum*+/+ and *Lum-/-* mice will be reciprocally transplanted to lethal gamma-irradiated mice. The inflammatory response of experimental mice will be

examined by epithelium debridement. The PMN isolated from experimental mice will be analyzed by chemotactic assay and infiltration to injured cornea. The *Lum*+/+ PMN transplanted to *Lum*-/- mice will develop phenotype of *Lum*-/- PMN and vise versa. The results will support the notion that Lum has a pivotal role in PMN formation.

Development of Gene Therapy Strategy for Treating Ocular Surface Diseases

When molecular cloning was introduced in early 1970's, gene therapy was highly touted as a possible method for curing congenital diseases caused by gene mutations. It has subsequently been hailed as the technology that will eventually lead to breakthroughs in the treatment of many acquired diseases such as cancer. Despite technological advances, however, few patients have benefited from gene therapy to date. Reasons for the limited success of gene therapy include difficulties of efficient gene transfer, inability to sustain and control expression of transgenes that leads to production of therapeutic quantities of proteins, and complications related to the introduction of foreign genes into humans.

The success of a gene therapy strategy relies on the delivery of reporter gene constructs to target tissues and sustained expression levels of the reporter genes sufficient to revert the pathological processes. Easy access of ocular surface tissues presents itself as an ideal model to examine the efficacy of gene therapy. We have adapted the techniques of adenovirus and intrastromal injection to deliver minigenes to corneas. We have recently demonstrated that application of AdenoSmad7 viral vectors to caused overexpression of Smad7 (an antagonist of TGF-b signaling) and effectively prevented ulceration of alkali-burned corneas. Thus, these techniques can be potentially used as a mean of gene transfer for ocular surface tissues, e.g., cornea, conjunctiva, eyelid for treating ocular surface tissue diseases.

Isolation and Characterization of Limbal Stem Cells

Limbal transplantation is an option of treating many ocular surface diseases. It is known that stem cells of corneal epithelium reside in the basal cell layer of limbus. Thus, the success of limbal transplantation to treat ocular surface diseases may result from the restoration of stem cells in the diseased tissues. However, our skill of culturing limbal stem cells is limited. Thus, there is a need to isolate and characterize the epithelial cells that posses characteristics of limbal stem cells, which can be used as un-limited sources for the use as auto graft or allograft to treat patients of limbal deficiency. We have recently used hTERT (human telomere reverse transcriptase) to establish an immortalized cell line from mouse stroma. Same technique will be used to establish immortalized human corneal epithelial cell line(s).

Can Bone Marrow Cells Differentiate and Become Corneal Epithelial Cells and Keratocytes?

It has been recently demonstrated that about 10-20% of corneal residential cells posses charactersitics of monocytes derived from bone marrow. Inflammation and corneal injury causes an inlux of bone marrow cells into the corneas. However, it remains unknown whether thses bone marrow derived corneal monocytes can differentiate and become cornea keratocytes and/or corneal epithelial cells. To examine these possibilities, *Kera-Cre* and *Krt12Cre/+* will be bred with ZEG reporter mice to produce *Kera-Cre/ZEG* and *Krt12Cre/+* bitransgenic mice. ZEG is a trangenic mouse line carrying floxed LacZ (flanked by loxP elements and enhanced green fluorescent protein (EGFP). *LacZ* gene is ubiquitously expressed by tissues that do not have Cre activity, whereaand s the excision of *LacZ* takes place in cornea of bitransgenic mice that express Cre driven by Kera and Krt12 promoters. The bone marrow cells will be isolated from the bitrnasgenic mice and transplanted to lethal gamma irradiated lumican and keratocan knockout mice. The recipient mice will be allowed to recover for 6-8 weeks and then be subjected to corneal epithelium debridement. During healing, monocytes will invade the cornea resulting from inflammatory response triggered by corneal injury. If the bone marrow cells differentaite and become corneal keratocytes

and epithelial cells, the LacZ gene will be excised and EGFP will be expressed by Cre from the donor bone marrow cells. The results will strongly support the hypothesis that bone marrow cells can differentiate and become corneal cell types, o.e., epithelial cells and keratocytes, suggesting that it is possible of using bone marrow cells to treat corneal limbal difficiency and corneal diseases involving keratocytes.