

University of Florida

Course GMS 5905/DEN 8290

RNA interference and microRNAs: from advances in cell biology to therapeutic applications in the treatment of human diseases

Schedule: all meetings in room D5-10 [Tuesday meetings noon-1pm and Friday meetings 2:30-3:30pm]

Date, time	Session/Topic	Paper presenters
First meeting Jan 15, 2013 Tuesday	<p>#1 Intro – RNAi basics siRNA and miRNA basics (Hyun Min Jung)</p> <p>Potent and specific genetic interference by double-stranded RNA in <i>Caenorhabditis elegans</i> http://www.nature.com/nature/journal/v391/n6669/full/391806a0.html</p> <p>The <i>C. elegans</i> heterochronic gene <i>lin-4</i> encodes small RNAs with antisense complementarity <i>lin-14</i> http://www.sciencedirect.com/science/article/pii/S009286749390529Y</p> <p>The widespread regulation of microRNA biogenesis, function and decay http://www.nature.com/nrg/journal/v11/n9/full/nrg2843.html</p> <p>MicroRNAs in rheumatoid arthritis http://www.sciencedirect.com/science/article/pii/S001457931100384X</p> <p>Crystal structure of <i>A. aeolicus</i> Argonaute, a site-specific DNA-guided endoribonuclease, provides insights into RISC-mediated mRNA cleavage http://www.sciencedirect.com/science/article/pii/S1097276505014759</p> <p>Disruption of GW bodies impairs mammalian RNA interference http://www.nature.com/ncb/journal/v7/n12/full/ncb1334.html</p> <p>Structural insights into the human GW182-PABC interaction in microRNA-mediated deadenylation http://www.nature.com/nsmb/journal/v17/n2/full/nsmb.1768.html</p>	
2 nd meeting Jan 18 Friday	<p>Methods in miRNA detection (Paul Dominguez)</p> <p>Northern Blot: http://www.idtdna.com/pages/products/mirna/starfire RealTime PCR Applied Biosystems Web Seminar http://appliedbiosystems.cnpq.com/lsc/webinar/russell/20070621/</p> <p>Microarrays Invitrogen (Life Sciences) NCode: http://www.invitrogen.com/etc/medialib/en/filelibrary/pdf.Par.44404.File.dat/B-073789_NCode_Brochure_1107.pdf Agilent Sure Print http://www.genomics.agilent.com/GenericB.aspx?PageType=Custom&SubPageType=Custom&PageID=2011 miRNA arrays: http://www.genomics.agilent.com/GenericB.aspx?pagetype=Custom&subpagetype=Custom&pageid=3216</p> <p>NextGen Seq Solid: http://marketing.appliedbiosystems.com/images/Product/Solid_Knowledge/flash/102207/solid.html Illumina http://seganswers.com/forums/showthread.php?t=21 Ion Proton Seq: http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Sequencing/Semiconductor-Sequencing/Semiconductor-Sequencing-Technology/Ion-Torrent-Technology-How-Does-It-Work.html</p>	
3 rd meeting Jan 22	<p>Practical example of miRNA study – Dereglated miRNAs in oral cancer (Hyun Min Jung)</p>	

Tuesday	miRNA-target mRNA prediction (EKL Chan) www.TargetScan.org miRBase: the microRNA database RNA22 http://cbcsrv.watson.ibm.com/rna22.html	
4 th meeting Jan 25 Friday	Introduction to RNAi therapy (EKL Chan)	
5 th meeting Jan 29 Tuesday	Viral miRNA and function (Rolf Renne, MGM)	
6 th meeting Feb 5 Tuesday Paper #1	Mukherji S, Ebert MS, Zheng GX, Tsang JS, Sharp PA, van Oudenaarden A. MicroRNAs can generate thresholds in target gene expression. Nat. Genet. 2011;43:854-9. <i>Learning objectives: current paper on miRNA-mRNA pathway</i>	Primary: Prema Secondary: Rachna Reader: Sunantha
7 th meeting Feb 22 Friday Paper #2 Paper #3	Ladewig E, Okamura K, Flynt AS, Westholm JO, Lai EC. Discovery of hundreds of mirtrons in mouse and human small RNA data. Genome Res, 2012;22:1634-45. Baccarini A, Chauhan H, Gardner TJ, Jayaprakash AD, Sachidanandam R, et al. (2011) Kinetic analysis reveals the fate of a microRNA following target regulation in mammalian cells. Current Biology 21: 369-376. <i>Learning objectives: miRNA processing and decay pathway</i>	Primary: Zirong Secondary: Prema Reader: Rachna Primary: Rachna Secondary: Zirong Reader: Lei
8 th meeting Feb 26 Tuesday Paper #4	Cambronne XA, Shen R, Auer PL, Goodman RH. Capturing microRNA targets using an RNA-induced silencing complex (RISC)-trap approach. Proc Natl Acad Sci U S A, 2012;109:20473-8. <i>Learning objectives: miRNA target identification</i>	Primary: Sunantha Secondary: Lei Reader: Zirong
9 th meeting March 12 Tuesday	Gene therapy using ribozyme and shRNA (Alfred Lewin, MGM)	
10 th meeting March 19 Tuesday Paper #5	Zisoulis DG, Kai ZS, Chang RK, Pasquinelli AE. Autoregulation of microRNA biogenesis by let-7 and Argonaute. Nature. 2012;486:541-4. <i>Learning objectives: miRNA biogenesis</i>	Primary: Lei Secondary: Sunantha Reader: Prema

11 th meeting March 26 Tuesday Paper #6	MicroRNA 218 Acts as a Tumor Suppressor by Targeting Multiple Cancer Phenotype-associated Genes in Medulloblastoma Sujatha Venkataraman, et al J. Biol. Chem. 2013 288: 1918-1928. <i>Learning objectives: tumor suppressor miRNA in cancer</i>	Primary: Sunantha Secondary: Zirong Reader: Prema
12 th meeting April 2 Tuesday Paper #7	Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, et al. (2011) Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci U S A 108: 5003-5008. <i>Learning objectives: miRNA as biomarkers</i>	Primary: Lei Secondary: Prema Reader: Sunantha
13 th meeting April 9 Tuesday Paper #8	Schulte LN, Westermann AJ, Vogel J. Differential activation and functional specialization of miR-146 and miR-155 in innate immune sensing. Nucleic Acids Res. 2013;41:542-53. <i>Learning objectives: miRNA in immunity</i> Review: O'Connell RM, Rao DS, Baltimore D. microRNA regulation of inflammatory responses. Annu. Rev. Immunol. 2012;30:295-312.	Primary: Zirong Secondary: Rachna Reader: Lei
14 th meeting April 16 Tuesday Paper #9	Ni X, Zhang Y, Ribas J, Chowdhury WH, Castanares M, Zhang Z, Laiho M, DeWeese TL, Lupold SE. Prostate-targeted radiosensitization via aptamer-shRNA chimeras in human tumor xenografts. J. Clin. Invest. 2011;121:2383-90. <i>Learning objectives: RNAi applications?</i> Reference: Zhou J, Rossi JJ. Aptamer-targeted RNAi for HIV-1 therapy. Methods Mol. Biol. 2011;721:355-71.	Primary: Rachna Secondary: Lei Reader: Zirong
15 th meeting April 23 Tuesday Paper #10	Lanford RE, Hildebrandt-Eriksen ES, Petri A, Persson R, Lindow M, Munk ME, Kauppinen S, Orum H. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science. 2010;327:198-201. <i>Learning objectives: neutralizing miRNA function in vivo</i> Review: Lindow M, Kauppinen S. Discovering the first microRNA-targeted drug. J. Cell Biol. 2012;199:407-12. PMID: 3483128.	Primary: Prema Secondary: Sunantha Reader: Rachna

Discussion format:

NIH study section style review-based discussion will be used. Each paper will be considered for its 1) overall scientific significance, 2) approach – methods and results, and 3) novel aspects etc. relative to our current concepts.

In brief, each paper will be assigned to three (or more) students/reviewers as “primary”, “secondary”, and “reader”. Individuals assigned as primary will take charge of the presentation and discussion and will spend no more than 25 minutes discussing critical issues in the selected paper using the guideline below. In order to be effective, some selection will have to be made... for example, not discussing every figure in equal details. **[Everyone is expected to read the paper ahead of time and participate in discussion.]** Individuals assigned as secondary will then spend 5 minutes to add points/comments not already discussed or take viewpoints different from the primary. Individuals assigned as reader will take brief notes/minutes/summary specifically related to strong positive/negative points/issues discussed during the meeting and submit a Summary within **72 hours**. Two examples for Summary are provided in the following pages.

On Reader’s Summary write-up:

The 1-2 page document should describe the general outline of the main findings of the paper we discussed and include comments about our discussion in class. Ideally it should serve as an editorial type document you read in a journal when someone discuss a new paper. After reading the editorial, readers should have some good ideas what this new paper is about including all the important points. A good editorial should serve to attract readers to read the new report in detail because it gives perspectives why the data are interesting and important. At the same time, readers should be aware of limitations of the new study as per our discussion.

Evaluation 2013

50% - Presentation of assigned paper - **Grades will be based on organization, clarity of presentations, ability to explain key data, consideration of Suppl. data provided, and other relevant information.**

Refer to Stanford’s Susan K. McConnell guidelines at iBioSeminars:

<http://www.youtube.com/watch?v=Hp7ld3Yb9XQ>

This is an outstanding presentation on how to present Powerpoint slides. Highly recommend to review it multiple times. The final powerpoint file is to be submitted for grades after presentation.

Additional points to discuss during presentation/discussion:

- Overall scientific significance of the paper being discussed – What is the perceived significance, do you agree with it?
- Have a slide on Approach, if appropriate - what type of methods were used to get the interesting results?
- For papers published in high impact journals, what do you think is novel aspects that allowed this paper to be accepted?

You may need to read up on the recent reviews listed in reference sections of the Outline.

30% - Written summary of discussions. See above. **Grades will be based on the quality of writing, ability to summarize key points during discussion, and additional insights applicable.**

Remaining 20% of the final grade will constitute active participation in Discussion. Attendance to all is required.

Other references:

Video:

General RNAi animation: <http://www.nature.com/nrg/multimedia/rnai/animation/index.html>

PAR-CLIP--a method to identify transcriptome-wide the binding sites of RNA binding proteins.

<http://www.ncbi.nlm.nih.gov/pubmed/20644507>

miRNA biogenesis and function

- Krol, J., Loedige, I. and Filipowicz, W. (2010) The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet*, **11**, 597-610.
- Bartel, D.P. (2009) MicroRNAs: target recognition and regulatory functions. *Cell*, **136**, 215-233.
- Fabian, M.R., Sonenberg, N. and Filipowicz, W. (2010) Regulation of mRNA translation and stability by microRNAs. *Annu Rev Biochem*, **79**, 351-379.
- Filipowicz, W., Bhattacharyya, S.N. and Sonenberg, N. (2008) Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet*, **9**, 102-114.
- Jakymiw A., et al. (2007). The role of GW/P bodies in RNA processing and silencing. *Journal of Cell Science* 120:1317-1323.

GW182 review:

- Eulalio, A., Triteschler, F. and Izaurralde, E. (2009) The GW182 protein family in animal cells: new insights into domains required for miRNA-mediated gene silencing. *RNA*, **15**, 1433-1442.
- Triteschler, F., Huntzinger, E. and Izaurralde, E. (2010) Role of GW182 proteins and PABPC1 in the miRNA pathway: a sense of deja vu. *Nat Rev Mol Cell Biol*, **11**, 379-384.

General Information

Course Director: Dr. Edward K.L. Chan

Office Location D5-22

E-mail address: echan@ufl.edu – preferred communication

Office Phone number: 273-8849

Office Hours: by appointment only - via email