

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: - Note all meeting in **D5-10**

Date, time	Session/Topic	Paper presenters
First meeting Sept 16 Tuesday noon to 1:30pm	#1 Intro – RNAi basics siRNA and miRNA basics (30-40 minutes - Songqing Li) Methods in miRNA detection (20 minutes - Paul Dominguez)	
2nd meeting Sept 19 Friday 2pm to 3:30pm	#2 miRNA and virus (40 minutes - Dr. Rolf Renne) Introduction to RNAi therapy (20 minutes - Dr. Andrew Jakymiw)	
3rd meeting Sept 23 Tuesday noon to 1:30pm	C. Y. Chu and T. M. Rana. Potent RNAi by short RNA triggers. <i>RNA.</i> , 2008. <i>Learning objectives: New 16nt siRNA? Are they really better than 20-23nt or 27-30nt siRNA? Implication for therapy? Off-target effect?</i> S. Guang, A. F. Bochner, D. M. Pavelec, K. B. Burkhart, S. Harding, J. Lachowiec, and S. Kennedy. An Argonaute transports siRNAs from the cytoplasm to the nucleus. <i>Science</i> 321 (5888):537-541, 2008. <i>Learning objectives: Novel Ago function? Nuclear RNAi function affecting RNAi-based therapy?</i>	Primary: Meghan O'Donoghue Secondary: Anitha Shenoy Reader: Paul Dominguez Primary: Dae In Kim Secondary: Paul Dominguez Reader: Anitha Shenoy
4th meeting Sept 30 Tuesday noon to 1:30pm	C. Addo-Quaye, T. W. Eshoo, D. P. Bartel, and M. J. Axtell. Endogenous siRNA and miRNA targets identified by sequencing of the Arabidopsis degradome. <i>Curr.Biol.</i> 18 (10):758-762, 2008. K. Okamura, W. J. Chung, J. G. Ruby, H. Guo, D. P. Bartel, and E. C. Lai. The Drosophila hairpin RNA pathway generates endogenous short interfering RNAs. <i>Nature</i> 453 (7196):803-806, 2008. Review: T. W. Nilsen. Endo-siRNAs: yet another layer of complexity in RNA silencing. <i>Nat.Struct.Mol.Biol.</i> 15 (6):546-548, 2008. <i>Learning objectives: What is endo-siRNA? How are they identified? What are their roles? How are they different than siRNA?</i>	Primary: Paul Dominguez Secondary: Meghan O'Donoghue Reader: Yong Hwan Kim Primary: Yong Hwan Kim Secondary: Paul Dominguez Reader: Songqing Li

<p>5th meeting Oct 7 Tuesday 12:30pm to 2:00pm</p>	<p>Turner, N.C., C.J. Lord, E. Iorns, R. Brough, S. Swift, R. Elliott, S. Rayter, A.N. Tutt, and A. Ashworth. 2008. A synthetic lethal siRNA screen identifying genes mediating sensitivity to a PARP inhibitor. <i>EMBO J.</i> 27:1368-1377. <i>Learning objectives: What is a synthetic lethal siRNA screen? What are the applications?</i></p> <p>R. Sandberg, J. R. Neilson, A. Sarma, P. A. Sharp, and C. B. Burge. Proliferating cells express mRNAs with shortened 3' untranslated regions and fewer microRNA target sites. <i>Science</i> 320 (5883):1643-1647, 2008. <i>Learning objectives: New regulation of miRNA and mRNA target? Must have implications for RNAi treatment of proliferating cancer cells?</i></p>	<p>Primary: Anitha Shenoy Secondary: Meghan O'Donoghue Reader: Yong Hwan Kim</p> <p>Primary: Paul Dominguez Secondary: Dae In Kim Reader: Md Abu Nahid</p>
<p>6th meeting Nov 4 Tuesday 12:30pm to 2:00pm</p>	<p>D. Baek, J. Villen, C. Shin, F. D. Camargo, S. P. Gygi, and D. P. Bartel. The impact of microRNAs on protein output. <i>Nature</i>, 2008.</p> <p>M. Selbach, B. Schwanhausser, N. Thierfelder, Z. Fang, R. Khanin, and N. Rajewsky. Widespread changes in protein synthesis induced by microRNAs. <i>Nature</i>, 2008. <i>Learning objectives: What is the real effect on miRNA on protein expression? Does it still make sense to use miR-based therapy?</i></p>	<p>Primary: Dae In Kim Secondary: Yong Hwan Kim Reader: Md Abu Nahid</p> <p>Primary: Yong Hwan Kim Secondary: Dae In Kim Reader: Meghan O'Donoghue</p>
<p>7th meeting Nov 7 Friday 2pm to 3pm</p>	<p>Gene therapy using ribozyme and shRNA (40 minutes - Dr. Alfred Lewin)</p>	
<p>8th meeting Nov 14 Friday 2pm to 3pm</p>	<p>P. Kumar, H. S. Ban, S. S. Kim, H. Wu, T. Pearson, D. L. Greiner, A. Laouar, J. Yao, V. Haridas, K. Habiro, Y. G. Yang, J. H. Jeong, K. Y. Lee, Y. H. Kim, S. W. Kim, M. Peipp, G. H. Fey, N. Manjunath, L. D. Shultz, S. K. Lee, and P. Shankar. T Cell-Specific siRNA Delivery Suppresses HIV-1 Infection in Humanized Mice. <i>Cell</i>, 2008.</p>	<p>Primary: Songqing Li Secondary: Md Abu Nahid Reader: Jennifer Kielczewski</p>
<p>9th meeting Nov 18 Tuesday noon to 1:30pm</p>	<p>J. Zhou, H. Li, S. Li, J. Zaia, and J. J. Rossi. Novel dual inhibitory function aptamer-siRNA delivery system for HIV-1 therapy. <i>Mol. Ther.</i> 16 (8):1481-1489, 2008.</p> <p>B. Huang, C. P. Mao, S. Peng, C. F. Hung, and T. C. Wu. RNA interference-mediated in vivo silencing of fas ligand as a strategy for the enhancement of DNA vaccine potency. <i>Hum. Gene Ther.</i> 19 (8):763-773, 2008. <i>Learning objectives: current examples of RNAi therapy?</i></p>	<p>Primary: Songqing Li Secondary: Md Abu Nahid Reader: Jennifer Kielczewski</p> <p>Primary: Jennifer Kielczewski Secondary: Yong Hwan Kim Reader: Dae In Kim</p>

<p>10th meeting Nov 21 Friday 2pm to 3pm</p>	<p>G. Shan, Y. Li, J. Zhang, W. Li, K. E. Szulwach, R. Duan, M. A. Faghihi, A. M. Khalil, L. Lu, Z. Paroo, A. W. Chan, Z. Shi, Q. Liu, C. Wahlestedt, C. He, and P. Jin. A small molecule enhances RNA interference and promotes microRNA processing. <i>Nat. Biotechnol.</i> 26 (8):933-940, 2008. <i>Learning objectives: small molecule influence on RNAi?</i></p>	<p>Primary: Jennifer Kielczewski Secondary: Md Abu Nahid Reader: Dae In Kim</p>
<p>11th meeting Nov 25 Tuesday noon to 1:30pm</p>	<p>S. Diederichs, S. Jung, S. M. Rothenberg, G. A. Smolen, B. G. Mlody, and D. A. Haber. Coexpression of Argonaute-2 enhances RNA interference toward perfect match binding sites. <i>Proc. Natl. Acad. Sci. U.S.A</i> 105 (27):9284-9289, 2008.</p> <p>P. Y. Chen, L. Weinmann, D. Gaidatzis, Y. Pei, M. Zavolan, T. Tuschl, and G. Meister. Strand-specific 5'-O-methylation of siRNA duplexes controls guide strand selection and targeting specificity. <i>RNA.</i> 14 (2):263-274, 2008. <i>Learning objectives: Is it feasible to increase RNAi efficiency and specificity?</i></p>	<p>Primary: Md Abu Nahid Secondary: Jennifer Kielczewski Reader: Anitha Shenoy</p> <p>Primary: Meghan O'Donoghue Secondary: Anitha Shenoy Reader: Jennifer Kielczewski</p>

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..

In brief, each paper will be assigned to three 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 the selected paper using the guideline below. In order to do this, 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 no more than 5-10 minutes to add points/comments not already discussed or take viewpoints different from the primary. Individuals assigned as reader will take brief notes/minutes specifically related to strong positive/negative points/issues discussed during the meeting. The summary will be emailed later to faculty who agree to serve as consultants to this discussion group. Any useful comment will be mentioned in subsequent meeting to provide feedback if applicable.

References for Introduction:

RNAi and siRNA basics

- *Fire et al.*, Potent and specific genetic interference by double-stranded RNA in *C. elegans* **Nature** 1998
- *Liu et al.*, Argonaute2 is the catalytic engine of mammalian RNAi **Science** 2004
- *Jakymiw et al.*, Disruption of GW bodies impairs mammalian RNA interference **NCB** 2005
- *Pillai et al.*, Inhibition of translational initiation by Let-7 MicroRNA in human cells **Science** 2005
- *Lian et al.*, Small Interfering RNA-mediated Silencing Induces Target-dependent Assembly of GW/P Bodies **MBC** 2007
- Review:

- Mello & Conte, Revealing the world of RNA interference **Nature** 2004
- Meister & Tuschl, Mechanisms of gene silencing by double-stranded RNA **Nature** 2004

miRNA biogenesis and function

- Du T. & P.D. Zamore (2005). microPrimer: the biogenesis and function of microRNA. *Development* 132: 4645-4652.
- Jakymiw A., et al. (2007). The role of GW/P bodies in RNA processing and silencing. *Journal of Cell Science* 120:1317-1323.

miRNA methods

USB (Northern)

<http://www.usbweb.com/mirtect-it/mirtectit-animation.html>

Applied Biosystems Web Seminar

<http://appliedbiosystems.cnpg.com/lasca/webinar/russell/20070621/>

Ambion microarray

<http://www.ambion.com/catalog/ProdGrp.html?fkProdGrp=372>

Exiqon

<http://www.exiqon.com/SEEEMS/10040.asp>

Kim et al., *TRENDS in Genetics*, 2006

Lu et al., *Nature*, 2005

RNAi therapeutics

Kim and Rossi, *Nature Reviews Genetics* (2007) 8,173-184.

de Fougerolles et al., *Nature Reviews Drug Discovery* (2007) 6, 443-453.

Aagaard and Rossi, *Advanced Drug Delivery Reviews* (2007) 59, 75-86.

Liu et al., *Histology and Histopathology* (2007) 22, 211-217.

Gonzalez-Alegre, *Pharmacology & Therapeutics* (2007) 114, 34-55.

Other general reviews:

Review on biochemical aspects of RNAi:

Paroo, Z., Q. Liu, and X. Wang. 2007. Biochemical mechanisms of the RNA-induced silencing complex. *Cell Res.* 17:187-194.

Review on RNAi therapy:

de, F.A., H.P. Vornlocher, J. Maraganore, and J. Lieberman. 2007. Interfering with disease: a progress report on siRNA-based therapeutics. *Nat. Rev. Drug Discov.* 6:443-453.

Kim, D.H. and J.J. Rossi. 2007. Strategies for silencing human disease using RNA interference. *Nat. Rev. Genet.* 8:173-184.

Gonzalez-Alegre, P. 2007. **Therapeutic RNA interference for neurodegenerative diseases: From promise to progress.** *Pharmacol. Ther.* 114:34-55.

2008 reviews:

L. Wu and J. G. Belasco. **Let me count the ways: mechanisms of gene regulation by miRNAs and siRNAs.** *Mol.Cell* 29 (1):1-7, 2008.

Filipowicz, W., S.N. Bhattacharyya, and N. Sonenberg. 2008. **Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight?** *Nat.Rev.Genet.* 9:102-114.

D. Haussecker. **The Business of RNAi Therapeutics.** *Hum.Gene Ther.* 19:451-462, 2008.