Disclosures: **J. Leheste:** None. **K.M. DiGregorio:** None. **E. Forbes:** None. **J.S. Honigman:** None. **V. Katz:** None. **B.H. Hallas:** None. **G. Torres:** None.

Poster

617. Molecular and Genetic Techniques

Location: Hall A-C

Time: Tuesday, November 15, 2011, 8:00 am - 12:00 pm

Program#/Poster#: 617.02/XX34

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: CIHR

HSFBC

Title: New method for protein knockdown: Targeted degradation of non-tagged protein via protein-protein interaction with ubiquitin-tagged protein.

Authors: ***T. W. LAI**[1,2], Y.-T. WANG[1];
[1]Univ. of British Columbia, Richmond, BC, Canada; [2]China Med. Univ. Hosp., Taichung, Taiwan

Abstract: The human genome project identifies approximately 20,000-25,000 genes in the human DNA, and this bio-diversity of gene-codes is further amplified by alternative mRNA splicing and post-translational protein modification. Nevertheless, the present techniques for nullifying genes and gene-products, such as DNA knockout and mRNA knockdown, are limited by (1) the inability to target specific mRNA splice-variants or modified proteins and (2) the inability to acutely study the function of long-lived proteins. Protein degradation in mammalian cells is largely via the ubiquitin-proteasome system, in which proteins destined for degradation is tagged with a signal protein called ubiquitin in a process called “ubiquitination” that signals its delivery for degradation by the proteasome. In studying the ubiquitin-based protein degradation, we found that non-tagged proteins (NTP) were also degraded via protein-to-protein interaction with an ubiquitin-tagged protein (UTP). This facilitated degradation of NTP was mediated by the proteasome, and was augmented when the dose of the UTP was incrementally increased. More importantly, UTP specifically knocked down NTP without affecting its alternative mRNA splice variant or other unrelated proteins. Truncation of the protein-protein interaction site, but not other unrelated sites, prevented facilitated NTP degradation. Single point-mutation of the interaction site, required for protein-protein interaction, also prevented facilitated NTP degradation. Like UTP, ubiquitin-tagged peptide (UTp) facilitated the degradation of NTP via direct peptide-protein interaction, and unlike UTP, UTp-mediated NTP degradation was not dose-dependent. Lastly, several shorter ubiquitin-fragments were constructed to facilitate the

synthesis of UTP and UTp for protein-knockdown experiments and clinical use. In conclusion, given the wide spread utilization of DNA knockout and mRNA knockdown protocols in research, this new method for direct protein knockdown will be of great interest to biology and medical researchers.

Disclosures: T.W. Lai: None. **Y. Wang:** None.

Poster

617. Molecular and Genetic Techniques

Location: Hall A-C

Time: Tuesday, November 15, 2011, 8:00 am - 12:00 pm

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Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: DFG GR 3619/2-1

DFG GR 3619/3-1

Title: Novel transgenic mice expressing inducible Cre recombinase under the control of the gonadotropin-releasing hormone promoter

Authors: *L. C. HOFFMANN[1], T. BUS[2], P. H. SEEBURG[2], V. GRINEVICH[2];
[1]Max-planck-Institute For Med. Res., Heidelberg, Germany; [2]Max-Planck-Institute For Med. Res., Heidelberg, Germany

Abstract: The hypothalamic gonadotropin-releasing hormone (GnRH), the central hormone of the hypothalamic-pituitary-gonadal (HPG) axis, is the principal regulator of fertility in all mammalian species. Binding of GnRH to gonadotropes of the anterior pituitary leads to the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which in turn regulate gonadal development and function. Several transgenic mouse lines constitutively expressing Cre recombinase or fluorescent markers under the GnRH promoter are currently available. We recently generated a new transgenic BAC mouse line, GnRH-CreERt2, expressing the fusion protein of a modified estrogen receptor ligand binding protein and a codon-improved Cre under the control of the GnRH promoter in an inducible manner. Immunohistochemical staining revealed that the Cre expression resembles the pattern of GnRH expression in the adult mouse brain, and virtually all GnRH neurons contain Cre immunoreactivity. To further verify the specificity of Cre expression in GnRH neurons, GnRH-CreERt2 mice were bred with Rosa26 reporter mice. Injections of tamoxifen, an estrogen receptor antagonist, which causes translocation of CreERt2 into the nucleus, induced the recombination of the Rosa locus, resulting in beta-galactosidase expression exclusively in GnRH neurons. However, vehicle (oil) injection