Defining the high affinity nicotinic receptor-associated proteome

Marina Picciotto
Depts. of Psychiatry, Neurobiology & Pharmacology
Yale University School of Medicine
Structure of nicotinic ACh receptors

acetylcholine

muscle type nicotinic receptor

neuronal type nicotinic receptors
Structure of nicotinic ACh receptors

Xie et al, Biol Psych, 2010
Nicotine does not stimulate dopamine release in β2 knockout mice

Grady et al, J Neurochem, 2001
...and does not support behaviors related to addiction

King et al, Neuropharm. 2004  
Brunzell et al, NPP. 2009  
Transgenic expression of $\beta_2$ in VTA rescues nicotine-induced locomotion (Mineur et al).

Viral-vector rescue of $\beta_2$ in VTA rescues nicotine self administration (Maskos, et al).

**Expression** of hypersensitive $\alpha_4$ or $\alpha_6$ nAChRs increases sensitivity to nicotine place preference (Tapper et al, Drenan et al).

**Knockout of** $\alpha_4$ in TH-positive neurons abolishes nicotine place preference (McGranahan, et al).

$\alpha_4/\alpha_6/\beta_2$ nAChRs in VTA are sufficient for nicotine reinforcement.
nAChRs involved in nicotine reinforcement

acetylcholine

muscle type nicotinic receptor

neuronal type nicotinic receptors
Polymorphisms in the \( \alpha 4 \) nAChR subunit

Xie et al, Biol Psych, 2010
Rare Nonsynonymous Variants in Alpha-4 Nicotinic Acetylcholine Receptor Gene Protect Against Nicotine Dependence

Pingxing Xie, Henry R. Kranzler, Michael Krauthammer, Kelly P. Cosgrove, David Oslin, Raymond F. Anton, Lindsay A. Farrer, Marina R. Picciotto, John H. Krystal, Hongyu Zhao, and Joel Gelernter

- All missense mutations appearing at conserved residues in the M3-M4 intracellular loop
- Cursory search of disrupted eukaryotic linear interaction motifs (ELM) narrowed focus
- Tested effects on: Receptor assembly/expression in HEK293 cells, Agonist-evoked responses in Xenopus oocytes, Interactome from immunoprecipitated receptor complexes
CHRNA4 Variants
Enrichment of phosphorylated proteins prior to LC-MS/MS protein ID effectively selects for mature pentamers vs retained intracellular intermediates.

Identified interactomes vary considerably across α4 rare variants.

α4P451L recruits importin isoforms and reduces 14-3-3 chaperone binding, yet no difference in nuclear fraction binding sites is found.

Additional variation in associated proteins is awaiting further validation with other model systems.
Defining the $\alpha_4\beta_2^*$ nAChR Interactome

- Studies of nAChR interactomes and regulation requires a quantitative, unbiased, high-throughput method for discovery-phase examinations.
- Integrating iTRAQ label-based quantitative proteomics with transgenic manipulation of the target protein.
Defining the $\alpha 4\beta 2^*\ nAChR$ Interactome

M270-immobilized mAb295:
- Produces near-complete capture of solubilized $\beta 2^*\ nAChRs$
- Optimal conditions are achieved with 5 $\mu g$ mAb/mg beads, used at 10% total sample volume.
Defining the $\alpha_4\beta_2^*$ nAChR Interactome

**Immunopurified $\beta_2^*$ nAChR Complexes:**

- Follow expected gene-dose expression
- Correlate well with iTRAQ quantitation
- Confirm earlier reports of subunit interdependence
Defining the $\alpha_4\beta_2^*$ nAChR Interactome

Initially identified 208 proteins:
- Frequency distribution was bimodal
- Indicated multiple processes involved

After correcting for cell compartment (based on UniProt assignment):
- List decreased to 98 proteins
- Unimodal distribution
Defining the $\alpha_4\beta_2^* nAChR$ Interactome

Further filtering based on correlation with internal standard ($\beta_2 nAChR$ subunit) yielded 17 proteins.
Defining the $\alpha_4\beta_2^*$ nAChR Interactome

What did we learn?

- iTRAQ sensitivity is equivalent to pharmacological methods for nAChR quantitation
- $\alpha_4$ and $\beta_2$ subunit expression is highly interdependent
- The majority of ID’d proteins did not follow linear association with $\beta_2$
- Low-abundance nAChR subunits ($\alpha_5, \alpha_6$) will require pre-enrichment for successful ID
Defining the $\alpha_4\beta_2^*$ nAChR Interactome

Perhaps most importantly:

- Coupling iTRAQ with gene-dose dependent expression of a target protein and immuno-affinity purification is a viable workflow for the ID of high-value targets for future study/validation.
\[ \beta_2^* \text{nAChR occupancy by [}^{123}\text{I}]\text{-A85380 is decreased in MD and BPD measured by In vivo SPECT} \]
nAChRs, Smoking, and Bipolar Disorder

Saturation of $[^{125}\text{I}]-A85380$ binding to postmortem tissue homogenates

Estimated $B_{\text{max}}$ and bound fmol at 200pM $[^{125}\text{I}]-A85380$ are equivalent

Degree of upregulation by smoking status hints at a discrepancy between Control and BPD
Control group: 127 proteins with significant smoking effect (51 up, 76 down)
BPD group: 135 proteins with significant smoking effect (50 up, 85 down)
59 proteins with significant BPD x Smoking interaction by ANOVA
Some proteins of note in control samples:
- 14-3-3 isoforms, CamKII and HSP variants are downregulated by smoking
- VILIP-1, NCAM1, synaptotagmin, and β-adducin are upregulated by smoking

Additional samples will augment and validate preliminary findings.
Future Aims

iTRAQ/nAChR transgenic project:
- Adding α4/β2 double-het group
- Cortical vs thalamic nAChRs
- Saline vs chronic nicotine groups

BPD nAChR project:
- Adding ‘n’ to label-free quantitation experiments
- Attempting stoichiometry estimations
Acknowledgments

Yale University
W.M. Keck Biotechnology Resource Laboratory
Kathy Stone
Chris Colangelo
Departments of Genetics and Psychiatry
Joel Gelernter

University of Pennsylvania
Jon Lindstrom
John Cooper

University of Colorado
Mike Marks
Sharon Grady