Annual Report of dr. Yi-Shin Lee
INCIPIT – Innovative Life Science PhD Program in South Italy

Period of activities: from 01/11/2017 to 31/10/2018

Reactivation of the dormant wild-type allele of MECP2 as a therapy for Rett syndrome:
screening of epigenetic compounds
Tutor: dr. Marcella Vacca

Main aim of the Research project
We will set up a cell-tool with specific Xi- and Xa- fluorescent-reporters allowing the
detection of Xi reactivation events at the Mecp2 locus in living cells. The dual reporter fluorescent
system will be used in the trial-screening of a small collection of epigenetic compounds (epiDrugs),
exploiting Mecp2-tagged mESCs and the Cellmak er automation workstation hosted at IGB. Allele-
specific autofluorescent reporters will serve as qualitative and quantitative readouts of the Mecp22-
reactivation assay.

Background
Heterozygous mutations in the MECP2 gene cause Rett syndrome (RTT; MIM 312750), a
severe neurodevelopmental disorder affecting 1:10000 girls and orphan of cure. Because MECP2 is
X-linked, its monoallelic expression depends on X-chromosome inactivation (XCI) pattern. As a
result, most female patients with RTT are somatic mosaic with approximately 50% of cells carrying
the wild type (WT) but silenced allele of MECP2 on the inactive (Xi) X chromosome. Proofs of
principle show that the stability of the silencing of X,-alleles can be pharmacologically or genetically
counteracted, by simultaneously targeting multiple players in the XCI maintenance. Currently, these
approaches lead to re-expression of several X-linked genes with severe side effects. Nonetheless,
the epigenetic reactivation of the MECP2 WT allele harbored by X,- could compensate for MECP2
deficiency within the mutant cells of patients, as a neuroprotective strategy.

Results obtained
To monitor the allele-specific expression of Mecp2, we are generating mice carrying a double
autofluorescent reporter system, where different tags are inserted within each allele of Mecp2
(XMecp2:eGFP /XMecp2:mCherry). Such system will be pivotal to specifically probe the reactivation of the
Mecp2l. Transgenic heterozygous females XMecp2:mCherry::LoxP /X (founders) with a mixed SV129/OLA
and C57BL/6j background were obtained in our lab last March. By the end of 2018, we will generate
XMecp2:mCherry::LoxP /Y males on a quite pure C57BL/6j genomic background.
In a first attempt to establish a reporter cell system for the in vitro reactivation assay, mouse
embryonic fibroblasts (MEFs) were isolated from XMecp2:eGFP /XMecp2 female embryos (co-supervisor,
dr. L. Casalino) and checked for a correctly balanced mosaicism. Nevertheless, due to the low
expression of Mecp2 in non-neuronal cells, the Mecp2:eGFP transgene-driven weak
autofluorescence made arduous the physical separation by FACS of the Mecp2:eGFP MEFS (reference cells) from the Mecp2:eGFP MEFs (test cells). Moreover, once sorted, the two
subpopulations were almost impossible to be detected and distinguished at the microplate reader
integrated to the Cellmaker, thus making reactivation events in Mecp2:eGFP MEFs impossible to
capture. To improve this issue, we decided to shift toward neural cells differentiated from mouse
embryonic stem cells (mESC) as an alternative.
During my first year of research training I obtained the following results:

1) In Western blot analysis (A) I show that recombinant female MEFs express very low levels of Mecp2 (both the endogenous and the recombinant protein) compared to mESC-derived neural progeny (NP) at day 13 post-plating (13dpp); 2) In qRT-PCR analysis (B) I verify that the insertion of the mCherry tag at C-terminus of MeCP2 protein does not interfere neither with the expression of Mecp2, nor with pluripotency and neural markers (not shown) in undifferentiated hemizygous Mecp2:mCherry male mESCs and in derived 13dpp NP; 3) If NP derived from hemizygous Mecp2:egFP and Mecp2:mCherry male mESCs are pooled together and then analyzed at FACS at 7dpp (C), in order to simulate the double-reporter female mESCs (to be derived), the two sub-populations of mCherry⁺ and egFP⁺ neural cells can be correctly detected and gated. Moreover, when these NP are re-seeded after sorting they are still able to differentiate towards mature neurons. 4) Most importantly, the autofluorescent signal of 13dpp Mecp2:egFP⁺ and Mecp2:mCherry⁺ NP (D) is suitable for a quantitative measurement at the microplate reader, a fundamental pre-requisite for the automated screening procedure (data not shown).

Research Skills and techniques acquired

1. MEF & ES cell culturing; 2. RNA, DNA and Protein purification; 3. Protein fractionation (nuclear vs cytoplasmic components); 4. Retrotranscription of RNA; 5. Western blot analysis; 6. Endpoint PCR; 7. qRT-PCR; 8. Immunolabelling; 9. living microscopy; 10. use of bioinformatic tools to characterize and manipulate nucleotidic sequences and for primers design; 11. participation in writing proposal for grants and fund-raising.

Communication and other skills acquired:

- Supervision and management of lab activities, protocols, papers on TRELLO.
- Focus on the meaning of what I want to communicate
- Maintain a positive attitude and smile, especially when I worked with Italian students from primary school.
- Use Neapolitan humor.
- Ask for helping when absolutely required.
- Minimize stress to effective communication (in progress).

Activities during the period concerned.

-Academic Activities: 60hr

-Research Conferences:
  - ICAN: Italian Conference on Autism and Atypical Neurodevelopment, Università degli studi di Napoli (complesso universitario Santi Marcellino e Festo, largo san Marcellino) 01.12.2017
  - ProRett meeting "RettSyndrome Research, Towards the future", Rome, 27-29.09.2018

-Training Activities:
• Communication Towards scientific community; How to write for biomedical journals and how to present scientific data at meetings., Naples, 23.03.2018
• Safety Issues for INCIPIT PhD Fellows, Naples, 24.04.2018
• Fundraising and attraction of private investment, Naples, 23.05.2018
• Industrial Property Rights: Patents, from R&D to market exclusivity, Naples, 10.07.2018
• Introducing our project to professor David Schlessinger, who is highly recognized for his pioneering work in basic molecular biology, including the mechanism of action of antibiotics and the application of human genetics and genomics to gain a better understand of diseases.

-Dissemination Activities:

• Outreach activities for primary school students: 19 March 2018, laboratory activities on DNA purification from fruit and bacteria cultures at Convitto Nazionale "V. Emanuele II, Napoli; 26 March 2018, laboratory activities on gel electrophoresis and microscopy at AREA di RICERCA Napoli 1.
• poster DREAM4 RTT: Drugs to REActivate MeCP2 for Rett syndrome, at the "RettSyndrome Research, Towards the future" meeting, Rome, 27-29.09.2018

Naples, 10/10/2018

Early Stage Researcher
Yi-Shin Lee