CHARACTERIZATION OF AN INDUCIBLE TRANSGENIC MOUSE MODEL FOR FRAGILE X-ASSOCIATED TREMOR/ATAXIA SYNDROME (FXTAS)

THESIS

A thesis submitted for the degree of Master of Biomedical Science majoring on Genetic Counseling

by
FATWA ADIKUSUMA
G4A008039

POST GRADUATE PROGRAM
DIPONEGORO UNIVERSITY
SEMARANG
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On April 13th, 2011
and has been approved by:

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DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgement is made in the text.

Semarang, April 2011

Fatwa Adikusuma

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Semarang, April 2011

Fatwa Adikusuma

LIST OF ABBREVIATIONS

ASO	: antisense oligonucleotides
(CGG)n	: CGG with (n) number of repeat
C57BL/6	: a type of mouse strain which is widely used for laboratory mouse
COS-7	: CV-1 (simian) in Origin, and carrying the SV40 genetic material line 7
DM	: myotonic dystrophy
DMPK	: myotonic dystrophiy protein kinase
dox	: doxycyline
eGFP	: enhanced green fluorescent protein
FISH	: fluorescence in situ hybridization
Fmr1	: fragile x mental retardation 1 (for animal)
FMR1	: fragile x mental retardation 1 (for human)
Fmrp	: fragile x mental retardation protein (for animal)
FMRP	: fragile x mental retardation protein (for human)
FXTAS	: fragile x-associated tremor/ataxia syndrome
Gapdh	: glyceraldehyde 3-phosphate dehydrogenase
GFA	: glial fibrillary acidic
GFP	: green fluorescent protein
GLAST	: glutamate–aspartate transporters
hnRNP	: Heterogeneous nuclear ribonucleoproteins

Hsp	: heat shock protein
IP	: intraperitoneal
KI	: knock-in
MBNL1	: muscleblind-like 1
MCP	: middle cerebellar peduncle
MRI	: magnetic resonance imaging
Pcp	: Purkinje cell protein
POF	: premature ovarian failure
PrP	: prion protein
RT-Q-PCR	: reverse transcriptase-quantitative-polymerase chain reaction
rtTA	: reverse-tetracycline transactivator
SCA	: spinocerebellar ataxia
Tet	: tetracycline
UTR	: untranslated region
ZNF9	: zinc finger protein 9

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ABSTRACT

Background: FXTAS (fragile X-associated tremor/ataxia syndrome) is a neurodegenerative disease with tremor and ataxia as the primary symptoms and ubiquitin-positive intranuclear inclusions as the major neuropathological hallmark. FXTAS affects premutation males with an expanded premutation CGG repeat in the promoter of the *FMR1* gene. The expanded premutation CGG RNA is considered toxic. Inducible transgenic mice were generated, using the Tet-on system to express transgene expanded premutation 99CGG along with an eGFP reporter under control of dox (doxycycline) induction, as well as specific driver promoter, the GFA2-rtTA and PrP-rtTA. This study aims to characterize those mouse models so they would be ready to be used for further studies.

Methods: Immunohistochemistry, Western blot, and RT-Q-PCR techniques were used to characterize the transgene expression of the transgenic mice. Characterization of ubiquitin inclusions formation used immunohistochemistry technique.

Results: The Tet-on-99CGG-eGFP transgene worked *in vivo*, while Tet-on-11CGG-eGFP lost its expression. Tet-on-99CGG-eGFP/GFA2-rtTA bigenic mice did not work *in vivo*, while Tet-on-99CGG-eGFP/PrP-rtTA bigenic mice did work. As expected, the Tet-on-99CGG-eGFP/PrP-rtTA mice formed the FXTAS hallmark, the ubiquitin-positive intranuclear inclusions after 12 weeks of dox induction.

Conclusions: Mouse model Tet-on-99CGG-eGFP/PrP-rtTA worked well, and were ready to be used for main further studies.

Keywords: FXTAS, *FMR1* gene, transgenic mice, Tet-on, PrP, GFA2, reversibility, ubiquitin inclusions

ABSTRAK

Latar belakang: FXTAS (*fragile X-associated tremor/ataxia syndrome*) merupakan penyakit neurodegeneratif dengan gejala utama tremor dan ataxia serta inklusi intranuklear ubiquitin yang muncul sebagai ciri-ciri utama secara neuropatologikal. FXTAS menyerang pria premutasi yang mengalami ekspansi repetasi CGG di promotor gen *FMR1*. RNA CGG premutasi dipercaya toksik. Tikus transgenik yang dapat diinduksi sudah dibuat, dengan menggunakan sistem Tet-on untuk mengekspresikan transgen 99CGG terekspansi bersama-sama dengan reporter eGFP, serta dapat dikontrol melalui induksi dox (doxycycline), juga menggunakan promotor pengendali yang spesifik yaitu GFA2-rtTA dan PrP-rtTA. Studi ini bertujuan untuk mengkarakterisasi tikus tersebut sehingga model tikus tersebut siap untuk digunakan untuk studi-studi berikutnya.

Metode: Teknik imunohistokimia, Western blot, dan RT-Q-PCR digunakan untuk mengkarakterisasi ekspresi transgene pada tikus transgenik. Karakterisasi inklusi ubiquitin menggunakan teknik imunohistokimia.

Hasil: Transgen Tet-on-99CGG-eGFP dapat bekerja secara *in vivo*, tetapi Tet-on-11CGG-eGFP kehilangan ekspresi transgennya. Tikus bigenik Tet-on-99CGG-eGFP/GFA2-rtTA tidak bekerja, sedangkan tikus bigenik Tet-on-99CGG-eGFP/PrP-rtTA dapat bekerja dengan baik. Sesuai harapan, tikus bigenik Tet-on-99CGG-eGFP/PrP-rtTA dapat menghasilkan inklusi intranuklear ubiquitin setelah induksi dox selama 12 minggu.

Kesimpulan: Model tikus Tet-on-99CGG-eGFP/PrP-rtTA dapat bekerja dengan baik dan siap untuk digunakan untuk studi penting berikutnya

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Kata kunci: FXTAS, gen *FMR1*, tikus transgenik, Tet-on, PrP, GFA2, reversibilitas, inklusi ubiquitin