THE USE OF MIFEPRISTONE (RU486) IN THE TREATMENT OF PSYCHOTIC MAJOR DEPRESSION

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Abstracts

The glucocorticoid receptor (GR) is an intracellular protein that is widely distributed throughout hippocampal and neocortical brain tissue. Mifepristone (RU486) is a potent GR antagonist that has also been shown to exhibit partial agonist-like effects. The precise location of the GR domain involved in the agonist-like activity of RU486 is unknown. Here, we examine this aspect of GR signaling by comparing human GR (hGR) construct with a Guyanese squirrel monkey GR (gsmGR) construct in which nuclear translocation and transactivation are known to be impaired. Using an objective translocation scoring method, we found that both hGR and gsmGR are translocated by RU486, and that nuclear translocation of hGR is significantly increased compared to gsmGR at 10 nM, 100 nM and 1000 nM RU486 in transiently transfected COS1 cells. While addition of RU486 to the cells transfected with hGR results in a 16-fold dose-dependent increase in transactivation compared to non-treated cells, no significant change in transactivation is observed with gsmGR at doses up to 100 nM RU486. Further experiments using six GR chimeras indicate that replacement of the hGR carboxyl-terminus of tau-1 transactivation domain (C-AF1, amino acids 132-428) with that from gsmGR diminishes hGR transactivation by RU486. These results demonstrate that RU486-induced transactivation of GR is determined in part by amino acids in the C-AF1 domain.

Introduction

Hyperactivity of the hypothalamic pituitary adrenal (HPA) axis in patients with major depression is one of the most consistent findings in biological psychiatry. These patients have been reported to release excessive amounts of glucocorticoid. The function of glucocorticoid is mediated by the glucocorticoid receptor (GR), a 94kD protein. GR is an intracellular receptor that, when unbound by ligand, exists in an inactivated state in the cytoplasm. Upon binding of the glucocorticoid ligand, the receptor is activated, translocated across the nuclear membrane, and is bound to specific palindromic DNA sequences in the promoter region of target genes. Dr. Alan Schatzberg's group has been conducting clinical trials treating acute psychotic depression patients with mifepristone and the results have been very successful. The steroid mifepristone, also known as RU486 (C-1073) is not only an antiprogesterone but also, at higher concentrations, an effective antagonist of glucocorticosteroid action in vitro and in vivo. Although mifepristone appears to substantially improve the psychotic and depressive symptoms seen in psychotic major depression, at molecular level the precise mechanism by which mifepristone represses the function of GR is not well understood. In this study we used hGR and gsmGR to seek a domain that determines RU486 repression to GR transactivation.

Results

Fig.1. RU486 differentially inhibits cortisol-induced GR transactivation by hGR and gsmGR.

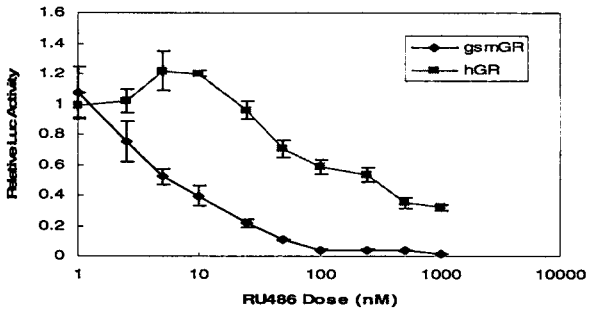


Fig. 2. RU486 antagonism is improved by replacement of the AF domain (amino acids 132-465) from gsmGR.

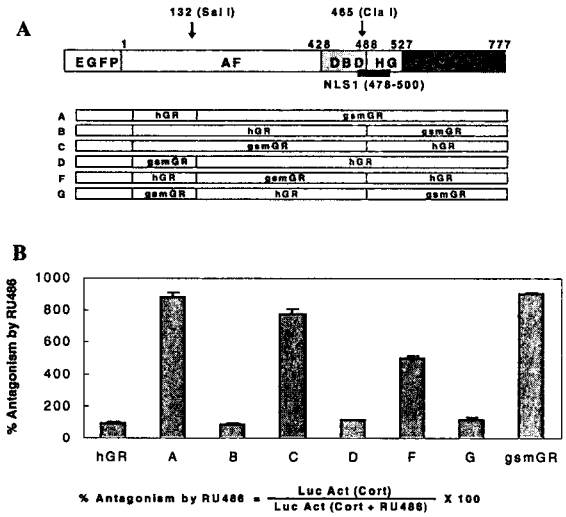
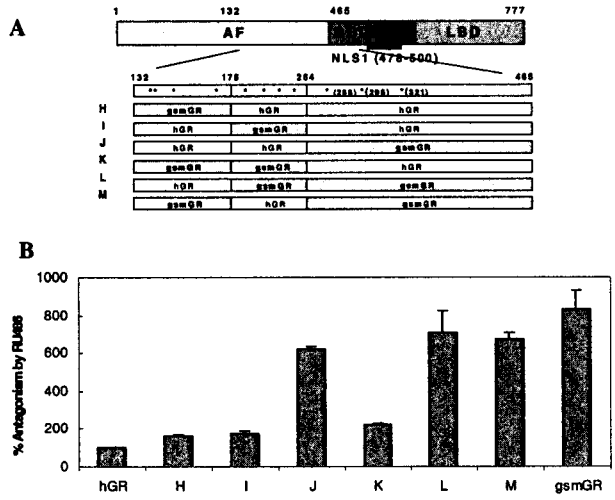


Fig. 3. RU486 antagonism is improved by replacement of an even smaller segment (amino acids 264-465) from gsmGR.



Conclusions

Results from our experiments showed that hGR and gsmGR exhibits a distinct RU486 repression to cortisol induced GR activation and furthermore a part in AF domain (Amino acid 264 – 465) of GR which contains three mutations is responsible for the RU486 response. Each three single mutations showed no change of RU response in the domain. We are investigating to conduct double and triple mutations from combination of the three single mutations to see whether these combinational mutants show a change of RU486 response. The projects will provide a fuller understanding of the underlying causes of depression and could lead to the development of new, more effective antidepressants through seeking genes that differentially interact with the two glucocorticoid receptors and investigating the mechanism of action of RU486.

Materials and Methods

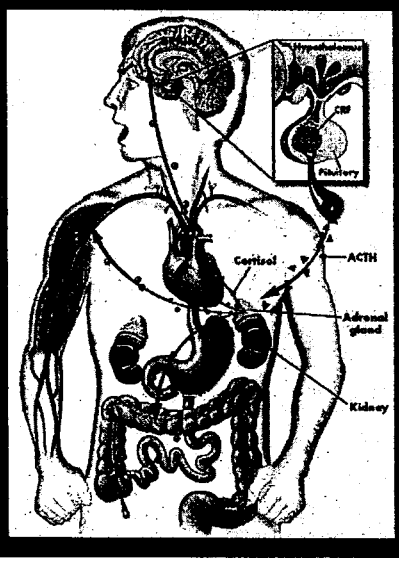
Cell Culture and Luciferase Activity Assay

COS1 cells (ATCC, Manassa, VA) cultured in DMEN with 10% fetal bovine serum in 5% CO2 at 37° C. Cells were cotransfected with pGRE-luciferase and pCMV-beta-gal-SPORT along with GR construct, using Superfect (Qiagen) at a ratio of 1:11:3 (GR construct:pGRE-luciferase:pCMV-beta-gal-SPORT). Cells were incubated for 12 hr and then treated with cortisol (Sigma, St Louis, MO) for 30h, and RU486 (Sigma, St Louis, MO) for 33h. Cell extracts were assayed for luciferase activity and beta-galactosidase activity. Data are presented as luciferase activity normalized to beta-galactosidase activity in the same transfection. Error bars represent + S.D. The effectiveness of RU486 was calculated with cortisol-treated luciferase activity divided by luciferase activity from treatment of both cortisol and RU486.

Chimeric Gene Construction with class IIS enzyme BfuA1

A. The homologous genes hGR and gsmGR are divided into three segments of the domain from amino acid 132 to 465 by PCR. B. In this example, the amplifications of hA and gsmB are shown. Each segment is amplified by PCR using a set of primers. The primers have a BfuA1 recognition site (boxed) upstream of an annealing sequence (underline). C. Following digestion with DpnI to destroy the template DNA, the fragments are digested with ClaI and BfuA1. In this example, only the hA-gsmB junction is shown. The digestion site of BfuA1 is away from the recognition site, and the digestion leaves gene-derived 4-bp overhangs (underline) unique to the junction. Following column purification, a desired combination of the digested DNA (e.g., gsmA, hB, gsm) is mixed and allowed to ligate.

스트레스



Rolling winds send a tree trunk and debris your way. Thankfully, your stress system helps you cope. The brain's hypothalamus releases the hormone corticotrophin-releasing factor (CRF) and its effects make your guard go up. CRF travels to the pituitary gland and triggers the release of adrenocorticotropic hormone (ACTH). This hormone travels in the blood to the adrenal glands and instructs them to release a third hormone, cortisol. The hormones rally the body systems and provide energy to help you deal with the stressful situation. You quickly flee. Perpetual or severe stress, however, may upset the stress system and harm the brain.

Physiological Response

Cortisol (Glucocorticoid)

Stimulation
- Protein catabolism
- Gluconeogenesis (liver)
- Triacyglycerol catabolism (adipose tissue)

Inhibition
- Insulin secretion (not brain)
- Inflammation and Immune response
- Digest tract

Adrenaline
- Cardiac output and Heart rate
- Ventilation
- Coagulability of blood

Diabetes
Ulcer
Cancer
Retarded growth

Effects on mood and behavior

Hypertension
Heart attack

Depression
Memory loss

Studies

I. Impaired Translocalization of monkeyGR

II. Differential GR antagonism between humanGR and monkeyGR

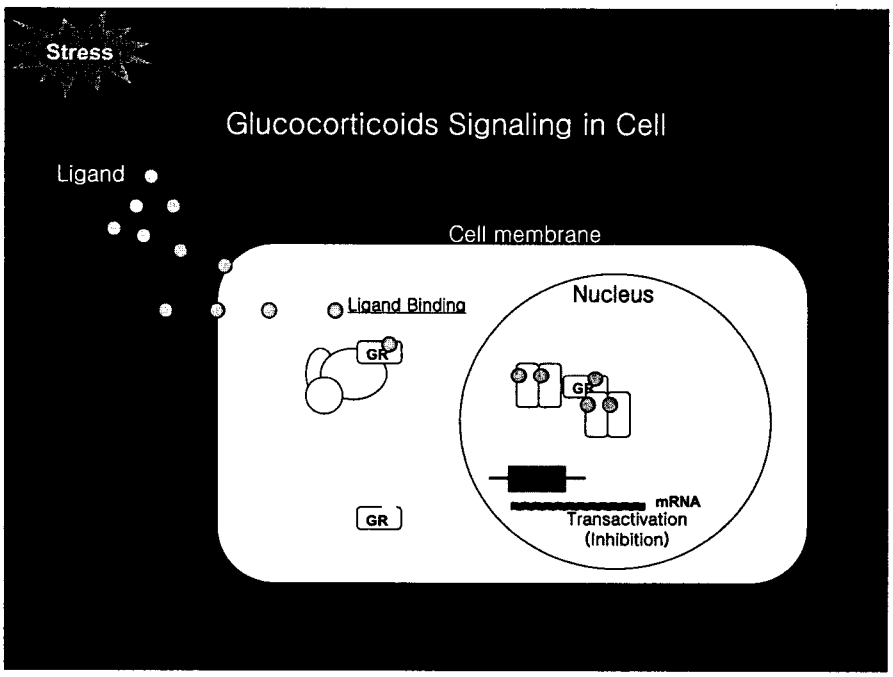
III. In vivo GR signaling

• Impaired Translocation of monkey GR

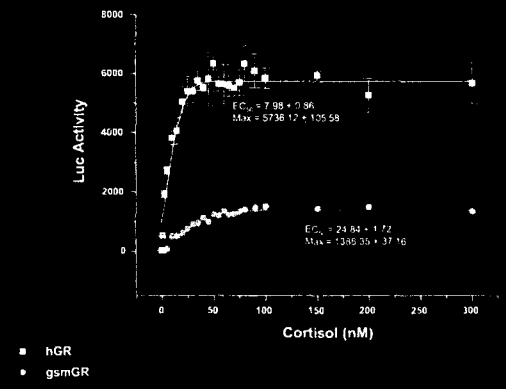
Malfunction in feedback regulation of the LHPA and cortisol hypersecretion is one of the most consistent findings associated with chronic stress (Depression). Squirrel monkey glucocorticoid receptors are insensitive to glucocorticoid mediated feedback regulation of the limbic-hypothalamic-pituitary-adrenal axis (LHPA).

Purpose

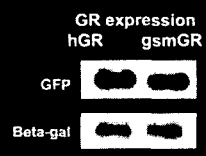
Identification of mutation in Squirrel monkey GR impaired to hGR, in an effort to understand the mechanism(s) for glucocorticoid feedback resistance.

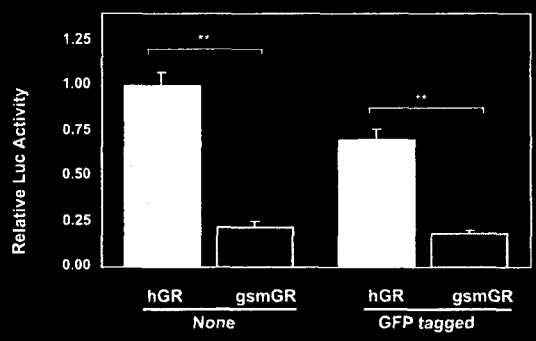


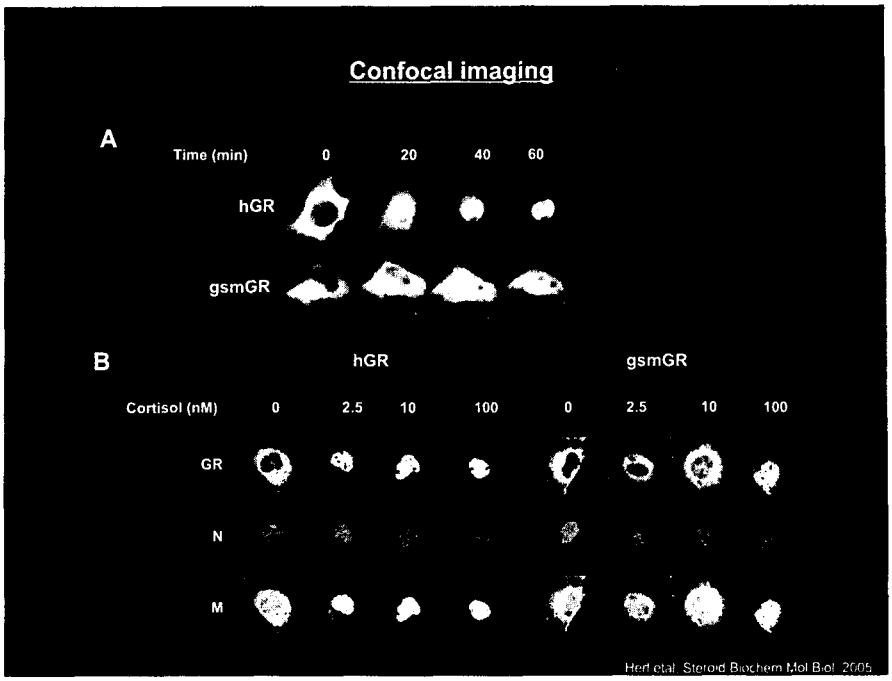
Impaired Transactivation of monkeyGR

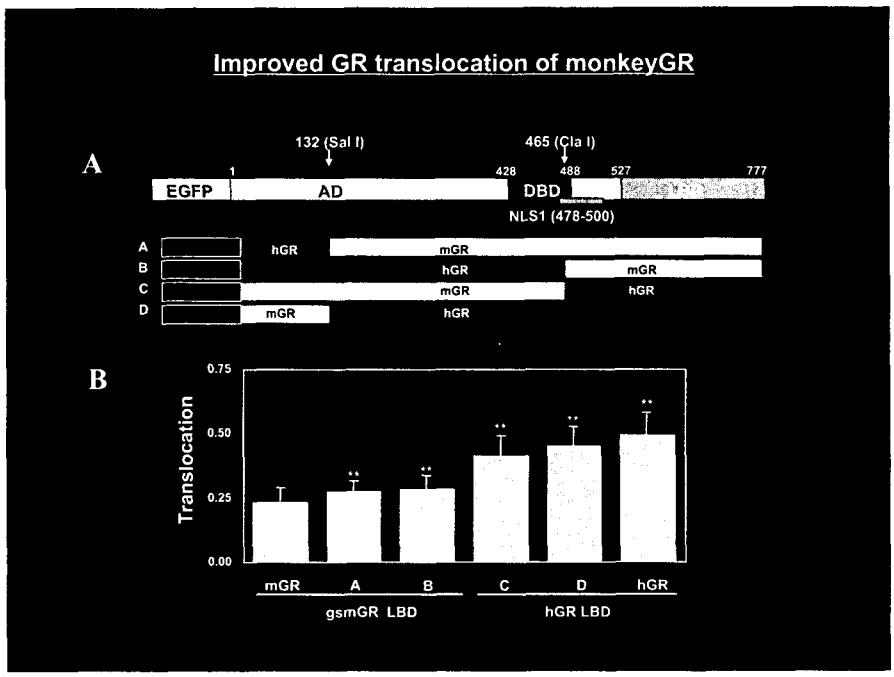


Reduced GR transactivation of gsmGR compared to hGR in both GFP-tagged and none tagged constructs

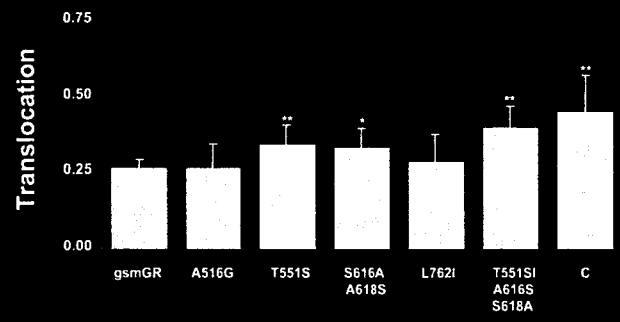




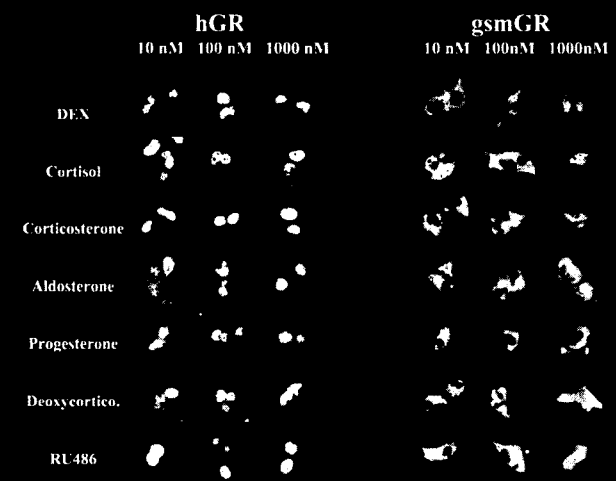

Confocal imaging
A
Time (min)
0
20
40
60
hGR
gsmGR
B
hGR
gsmGR
Cortisol (nM)
0
2.5
10
100
0
2.5
10
100
GR
N
M
Herd etal. Steroid Biochem Mol Biol. 2005


Improved GR translocation of monkeyGR
A
132 (Sal I)
465 (Cla I)
1
428
488
527
777
EGFP
AD
DBD
NLS1 (478-500)
A
B
C
D
hGR
mGR
B
Translocation
0.75
0.50
0.25
0.00
mGR
A
B
C
D
hGR
gsmGR LBD
hGR LBD

Recovery of Translocation by Mutations in the LBD



Translocation of GR by various hormones



II. Differential antagonism between hGR and mGR

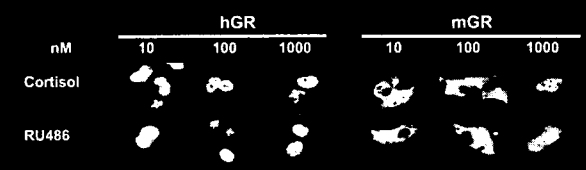
Preliminary trials suggest that the controversial drug RU-486 (mifepristone) provides sudden relief for psychotic depression, a disease normally very difficult to treat. But since progesterone receptors and cortisol receptors are structurally related, mifepristone also blocks progesterone, an effect that makes it useful as abortion and, in smaller doses, as an emergency contraceptive.

Purpose

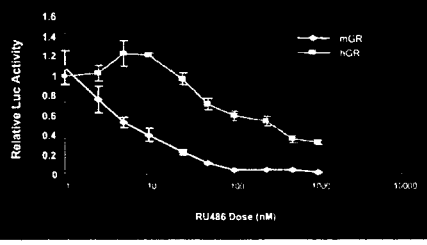
Identification of GR domain in the monkey GR showing higher antagonism compared to hGR, in an effort to screen drugs targeting to proteins involved in specific GR antagonism for psychotic depression treatment.

Differential RU486 repression to GR activity between hGR and mGR

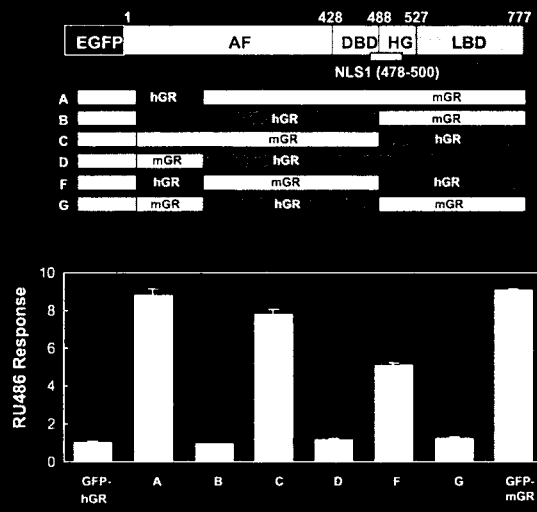
A. Representative fluorescence images of GR translocation



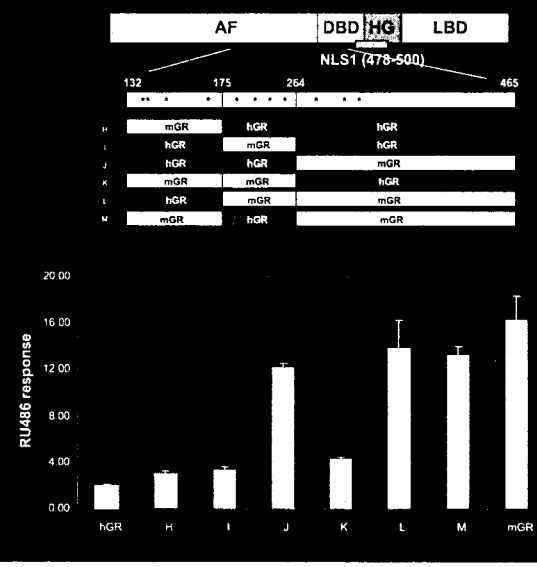
B. Differential RU486 repression to GR activity between hGR and gsmGR



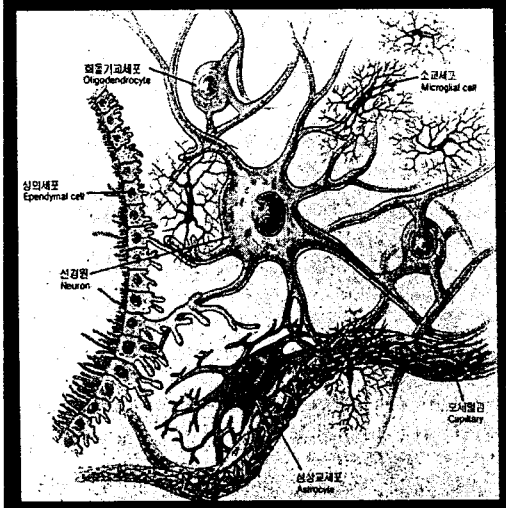
Improved RU486 response of hGR by replacement of AF domain (Amino acid 132-465) from gsmGR



Effect of amino acid 264-465 in AF domain (amino acid 264-465) on RU486 response



III. in vivo GR signaling



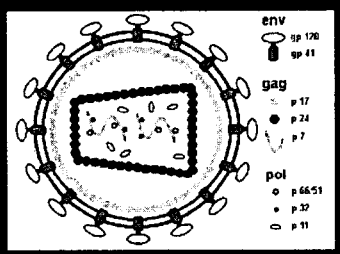
1. Astrocytes 성상교 세포 (nurse cell)
- regulate environment, K+, glucose, remove neurotransmitter
- support-physical, nutritional and ionic, barrier function
- nutrition transfer- from capillary to neurons
- divide rapidly (source of fast growing tumors)
- stain by GFAP (glial fibrillary acidic protein)

2. Microglia 소교세포 (packman)
- brains relative of monocyte-blood cells that become major packman in different tissue types
- phagocytes, clean up debris
- stain by CR3C (monoclonal CD116)

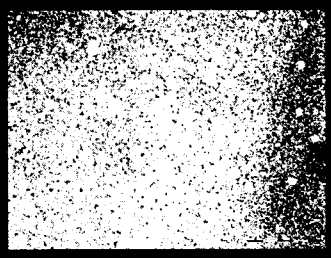
3. Olicodendrocytes 희돌기세포
- myelinate axons

4. Ependymal cells 상의세포
- lining cells of the ventricular system (lines lateral ventricle)
- permeable lining
- produce CSF proteins, secretion

Lentivirus



Lentivirus (lenti-, Latin for "slow") is a genus of slow viruses of the Retroviridae family, characterized by a long incubation period. Lentiviruses can deliver a significant amount of genetic information into the DNA of the host cell, so they are one of the most efficient methods of a gene delivery vector.



TEM image - Lentivirus particles (pWPXL Ins 5X GRE mRFP) captured at KBSI (Oh, JM), 2007 Particle size 20~30nm

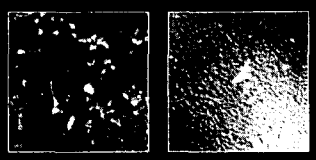
Why does the Lentiviral vector choice?

1. The transfection efficiency using Lentiviruses is high.
2. Lentiviruses can infect non-dividing cells, dividing cells and furthermore animal.
3. Lentiviral vectors have proven to be effective without toxicity and immune responses.
4. Long expression period (around 1 year)

Application

1. Detection of signaling pathway in in vivo animal system
2. Identification of novel ligands for specific Transcription Factors
3. Screening for agonists and antagonists of specific ligands/receptors
4. Tracing stem cells

A. Standard Lentivector

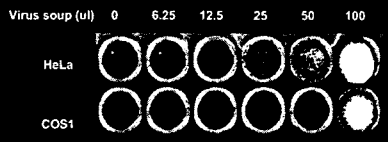


Dilution 1 X 10 X 100 X

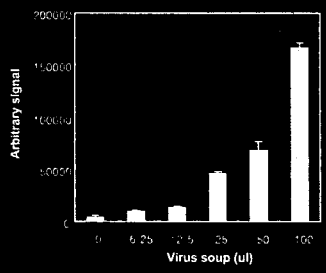
Plating of 30,000 HeLa cells per 24-well plate (500ul virus soup). Infection the Hela cells with 500ul of 10-fold serial Diluted lentivirus soup (GFP). Fixation and Detection of GFP infected Hela cells by Confocal microscope.

B. Bicistronic Lentivector (Luciferase, dsRed2)

1. Luciferase – IVIS200



Luciferase signal (HeLa cells)



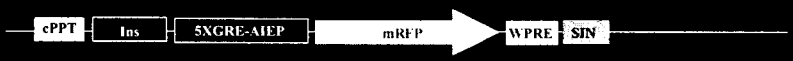
2. dsRed2 – MP Confocal Microscope

HeLa COS1

C. Cortisol inducible Lentivector

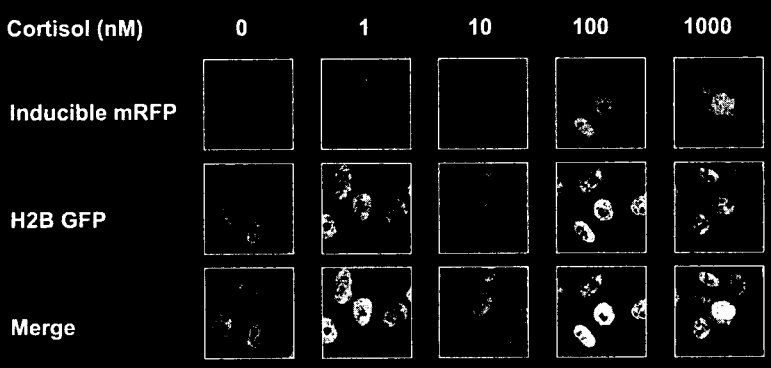
1. pWPXL Ins 5XGRE AIEP mRFP

2. pWPXL Ins 5XGRE AIEP Luc

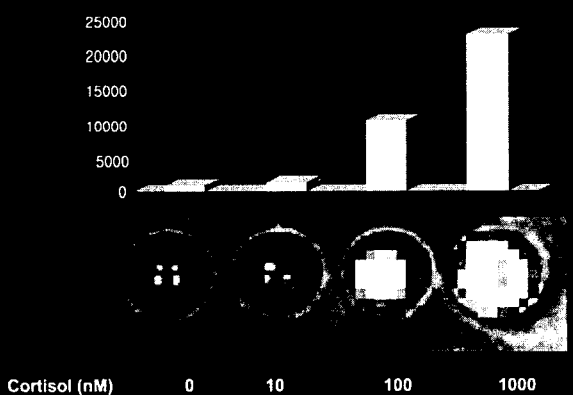


3. pWPXL EF1a GFP Ins 5XGRE AIEP mRFP

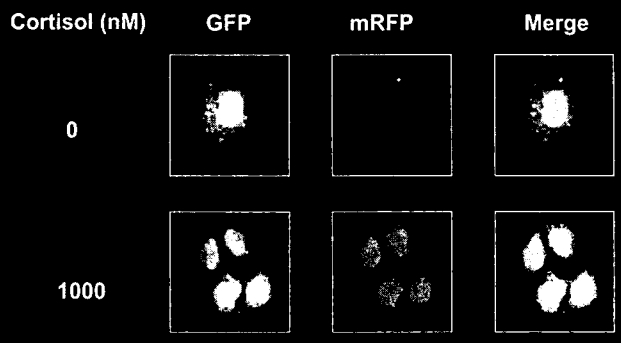
1. Dose dependent mRFP expression



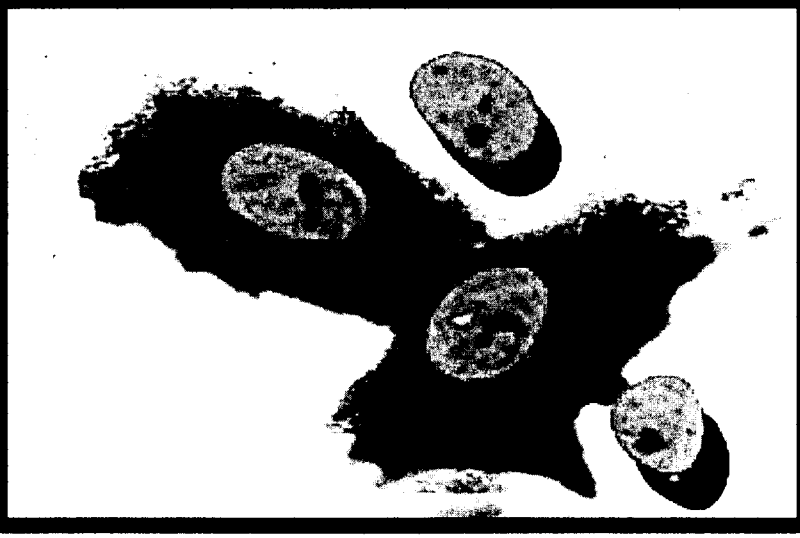
2. Dose dependent Luciferase expression



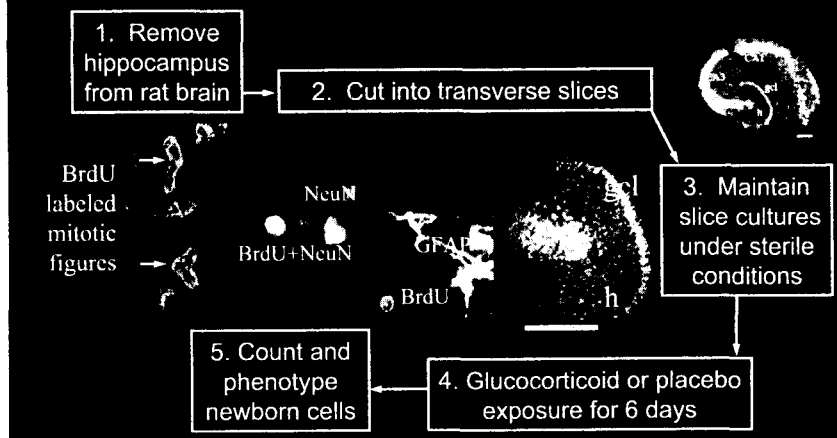
3. Duel expression lentivector



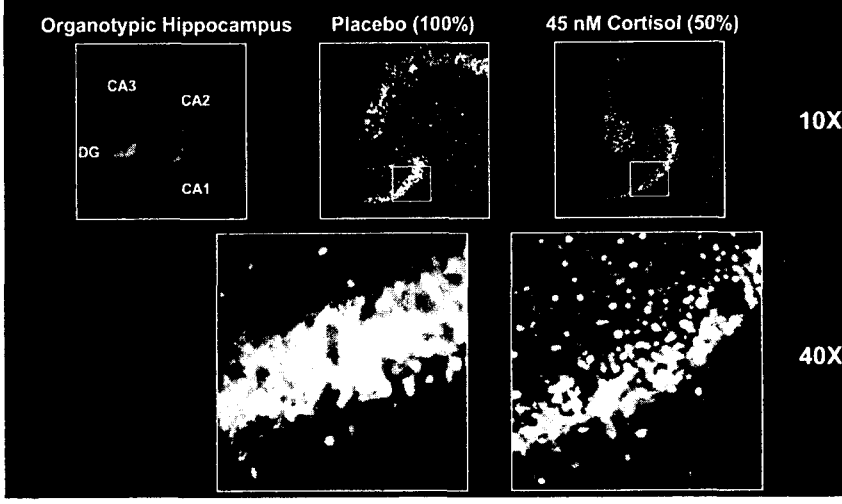
mRFP in HeLa Cells



Experimental Studies of Rodent Slice Cultures



Glucocorticoid Impaired Neurogenesis in Hippocampus



Stress, Depression, and Brain Atrophy

New Targets for Therapeutic Interventions