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Forum Minireview

Nuclear Receptors as Targets for Drug Development: The Role of Nuclear Receptors During Neural Stem Cell Proliferation and Differentiation

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Abstract. The fate of stem cells, such as neural stem cells and hematopoietic stem cells, depends on strictly regulated signaling events including activation of nuclear receptors, resulting in subsequent gene induction. Recently, we demonstrated that PPARγ, a ligand-activated nuclear receptor, plays an important role in regulating the proliferation and differentiation of murine neural stem cell (NSC). NSC prepared from heterozygous PPARγ-deficient mouse exhibited a slower growth rate compared with that of wild-type mouse, which was also demonstrated in PPARγ-knockdown NSC that was generated by the lentiviral-vector-mediated RNA interference approach. These studies have important implications for understanding central nervous system functions and developing a therapy for neurodegenerative disorders. In this review, recent findings on stem cell biology, especially focusing on nuclear receptors in NSCs, including our current study, will be discussed.

Keywords: neural stem cell, nuclear receptor, peroxisome proliferator-activated receptor (PPAR) γ

Introduction

The area of stem cell research has been marked by many unprecedented advances, and much of the attention in the field of regenerative medicine and others has been focused recently on stem cells. Stem cells are undifferentiated cells with the capacity for unlimited or prolonged self-renewal and the ability to give rise to differentiated cells. In an effort to seek stem cells in diverse tissues, researchers have succeeded in identifying stem cells (possible stem cells) in the hematopoietic system (1), central nerve system (2, 3), liver (4), intestine (5), pancreas (6), skin (7), hair follicle (8), etc., implying that cells fulfilling the minimal definitions of "stemness" that is self-renewal and multipotency might be present in virtually all tissues and most stages of development.

Neural stem cells (NSCs) are one of the most attractive cells for an application of neural transplantation or drug discovery for neurodegenerative diseases such as Parkinson's disease, Huntington's disease, nerve injury, stroke, and multiple sclerosis (9) because they exhibit several important and potential advantage features: i) NSCs are easy to isolate from embryonic or adult brain and expand in free-floating "neurosphere" cultures in the presence of epidermal growth factor (EGF) and/or basic fibroblast growth factor (bFGF) (2, 3, 10); ii) these cultured cells are able to differentiate and form the three terminal functional cells of the nervous system, which are astrocytes, oligodendrocytes, and neurons. Therefore, many studies have been performed to investigate the mechanisms involved in the proliferation and differentiation of NSCs (11 – 13). There are, however, remarkably few articles on nuclear receptor function in NSC. In this review, we focused on NSCs and discuss the possibility that stem cell fate may be critically regulated by some of the nuclear receptors, along with the results obtained so far in our research concerning peroxisome proliferators-activated receptor (PPARγ).

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ERs-dependent regulation of NSCs

The estrogen receptor (ER) α and the more recently discovered ERβ belong to the nuclear hormone receptor superfamily. Estrogen has effects on sexual differentiation and reproduction, but can also alter brain structure and functions that may contribute to Alzheimer's disease and cerebral stroke (14, 15). Brannvall et al. reported that embryonic and adult rat NSCs express ERα and ERβ, with higher levels of ERβ, and the activation of ERs decreased the proliferation of NSCs in the presence of EGF (16). Furthermore, upon differentiation into neurons and glial cells, estrogen treatment increased the number of β-tubulin-positive neurons. The results obtained using a receptor antagonist showed that the effects of estrogen on the differentiation of NSCs involve activation of ERs. Consistent with the higher levels of ERβ in NSCs, the brains of ERβ knockout mice show several morphological abnormalities such as a severe neuronal deficit and a remarkable proliferation of astroglial cells in the limbic system (17), while no major abnormalities were observed in the brain of mice carrying a null mutation for the ERα. These phenomena suggest that ERs, especially EFβ, signaling pathways contribute to NSCs differentiation rather than its self-renew.

TLX-dependent regulation of NSCs

TLX (a mouse homolog of the *Drosophila tailless* gene) was initially identified as an orphan nuclear receptor expressed in the invertebrate (18, 19) and vertebrate forebrain (20), and it is highly expressed in the adult brain (21). TLX-null mice are grossly normal at birth, indicating that TLX is not required for prenatal survival, but show impaired development of a specific subset of forebrain-derived structure, suggesting that the TLX gene is required for the proliferation or survival of a subpopulation of neural progenitors in adult mice (22). Evans's group recently found that TLX-expressing cells isolated from adult brain comprise a self-renewing population and these cells have the ability to give rise to all three neural cell types upon differentiation, indicating that TLX-expressing cells are multipotent (23). Immunostaining for nestin (a common marker of proliferating CNS progenitors) showed that whereas TLX-expressing (TLX+/−) cells are nestin-positive, the TLX−/− cells are nestin-negative. Interestingly, restoration of TLX in TLX−/− cells shows cell proliferation and increased expression in nestin with silencing of the astrocyte marker GFAP. These data imply that TLX may be one of the key regulators that act by controlling the expression of a network of target genes to establish the undifferentiated and self-renewable state of NSCs.

PPARγ-dependent regulation of NSCs

PPARγ plays a central role in adipocyte differentiation and insulin sensitivity. Moreover, recent observations reveal that PPARγ has diverse effects upon other cell types and organs. For example, we and others have previously reported that endogenous PPARγ provides anti-inflammatory activity against inflammation animal models (24) and suppression of colon carcinogenesis (25). These reports suggest that PPARγ and its related pathways play an important role in regulating cellular differentiation and tissue homeostasis (26, 27). Recently, we demonstrated that optimal activation of the PPARγ pathway stimulated NSC proliferation and inhibited differentiation of NSCs into neurons (ref. 28 and unpublished data). In contrast, the PPARγ antagonist bisphenol A diglycidyl ether (BADGE) inhibited cell growth. PPARγ was strongly expressed in the embryonic brain, but hardly detected in the newborn and adult brain. Consistent with these observations, a high level of PPARγ expression was detected in cultured NSCs isolated from embryo. Notably, NSCs prepared from heterozygous PPARγ-deficient mouse exhibited a slower growth rate compared with that of wild-type mouse. To confirm the crucial rule of PPARγ in NSCs, we have applied the RNA-interfering approach. We have previously established a lentivirus-mediated short hairpin RNA expression system (LV-shRNA) and identified a potent short hairpin RNA that suppresses PPARγ expression, resulting in marked inhibition of preadipocyte-to adipocyte differentiation in 3T3-L1 cells (Fig. 1) (29). In the case of LV-shRNA-Lu or -P18, which is unable to suppress the expression of PPARγ protein, the proliferation of NSCs was not altered by LV-shRNA infection. In contrast, NSCs infected with LV-shRNA-P15 or -P17, which can suppress PPARγ expression, exhibited the significant decrease in cell growth rate (Fig. 2). Although PPARγ-dependent mechanisms controlling NSCs proliferation and differentiation remain unclear, our observations indicate that PPARγ plays an important role during the early stages of development of the central nervous system.

Null1-dependent regulation of NSCs

Null1 (also known as NR4A2), an orphan nuclear receptor lacking identified ligands, is specifically required for the induction of midbrain dopaminergic neurons, which fail to develop in Nurr1-null mutant mice (30). Homozygous mice are born without apparent abnormalities, but died within the first 2 days after birth

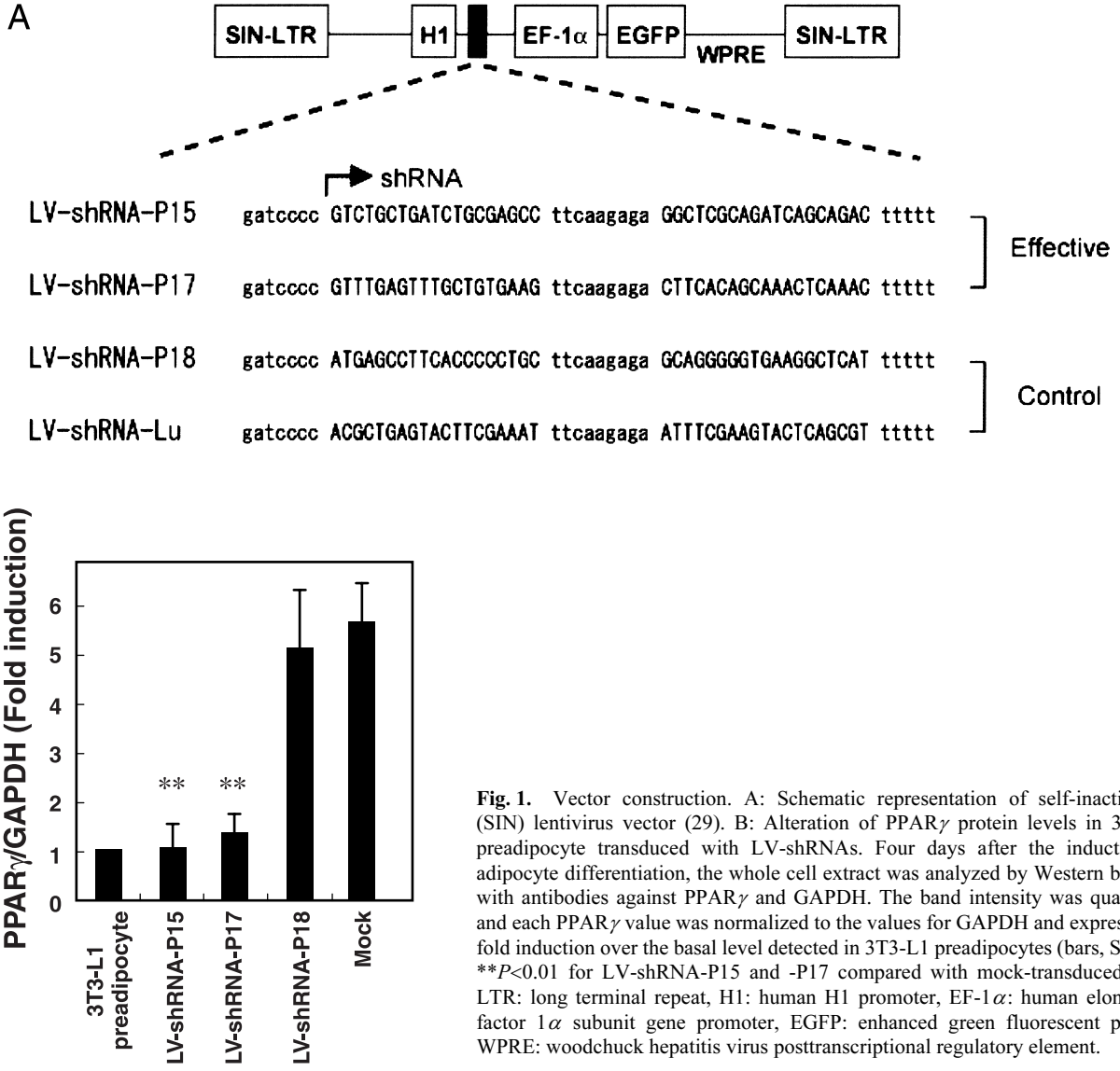

Fig. 1. Vector construction. A: Schematic representation of self-inactivating (SIN) lentivirus vector (29). B: Alteration of PPARγ protein levels in 3T3-L1 preadipocyte transduced with LV-shRNAs. Four days after the induction of adipocyte differentiation, the whole cell extract was analyzed by Western blotting with antibodies against PPARγ and GAPDH. The band intensity was quantified and each PPARγ value was normalized to the values for GAPDH and expressed as fold induction over the basal level detected in 3T3-L1 preadipocytes (bars, S.E.M). $^{**}P<0.01$ for LV-shRNA-P15 and -P17 compared with mock-transduced cells. LTR: long terminal repeat, H1: human H1 promoter, EF-1α: human elongation factor 1α subunit gene promoter, EGFP: enhanced green fluorescent protein, WPRE: woodchuck hepatitis virus posttranscriptional regulatory element.

due to inability to suckle. Adult heterozygote also manifested reduced striatal dopamine levels but no apparent histological or behavioral abnormalities, indicating that Nurr1 helps maintain the differentiated dopamine neuron phenotype. Kim et al. also demonstrated that Null1 restricts uncommitted multipotent mouse NSCs to neuronal lineage in the presence of fibroblast growth factor-8 and sonic hedgehog signaling (31). Consequently, it was expected that a putative ligand for Nurr1 may provide opportunities for pharmacological intervention to manage the function of dopaminergic neurons in neurodegenerative diseases.

A year ago, Wang et al. reported a surprising finding concerning the structure of the Nurr1 ligand-binding domain (LBD) by X-ray crystallography: the Nurr1 LBD contains no cavity as a result of the tight packing of side chains from several bulky hydrophobic residues in the region normally occupied by ligands; Nurr1 lacks a classical binding site for coactivators (32). Notably, however, Wang et al. also demonstrated that transcriptional activity of Nurr1 is correlated with regulated, ligand-independent stabilization of the Nurr1 LBD. These findings point to additional complexities in the transcriptional regulation of target genes by nuclear receptors, but imply that they play important biological roles in the tissues in which they are expressed.

Concluding remarks

Is there any correlation among these nuclear receptors that were discussed above? It is well known that nuclear receptors can interact with other receptors, such as

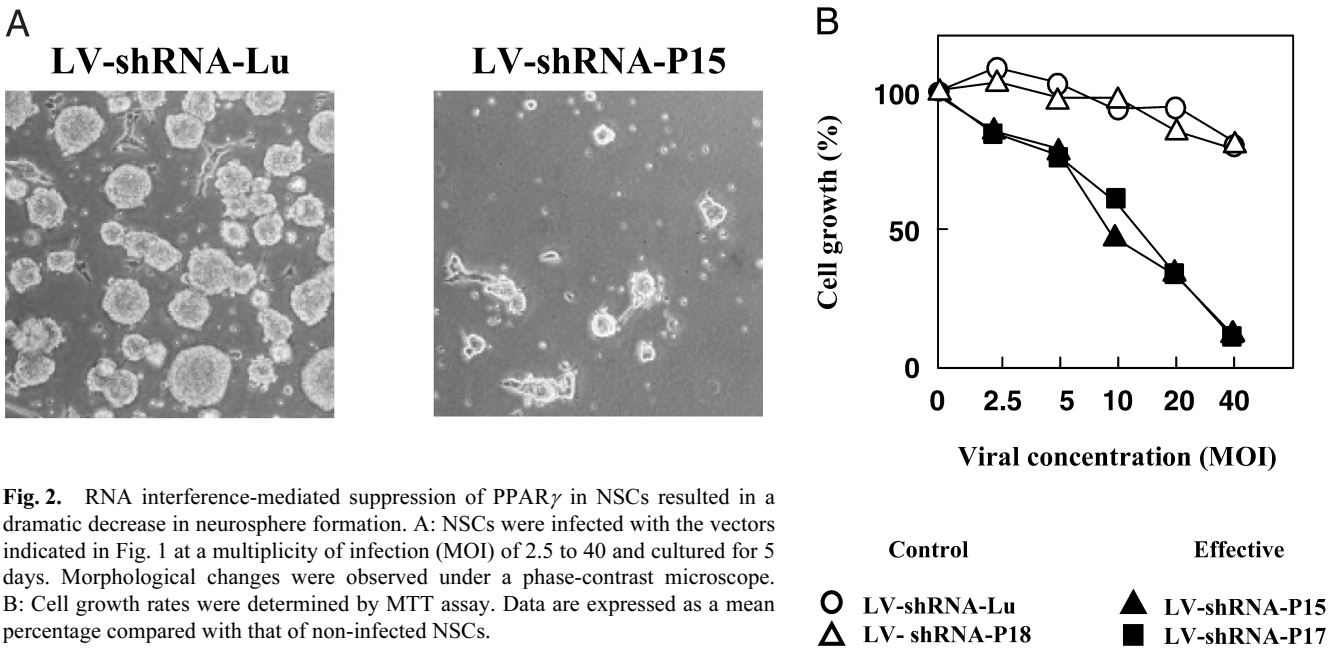

Fig. 2. RNA interference-mediated suppression of PPARγ in NSCs resulted in a dramatic decrease in neurosphere formation. A: NSCs were infected with the vectors indicated in Fig. 1 at a multiplicity of infection (MOI) of 2.5 to 40 and cultured for 5 days. Morphological changes were observed under a phase-contrast microscope. B: Cell growth rates were determined by MTT assay. Data are expressed as a mean percentage compared with that of non-infected NSCs.

retinoid X receptors (RXRs). Nuclear receptors can also recruit subsets of transcriptional coactivator proteins to the receptor complex. Thus, these phenomena that were observed in NSCs could be expressed in part through the interaction with other proteins. Nurr1 can interact with the target DNA as monomers, homodimers, or heterodimers with RXRs, and PPARγ can form a heterodimer with RXRα. It is possible, therefore, that the activation of PPARγ causes the suppression of Nurr1 transcriptional activity through competitive binding with RXR, resulting in the inhibition of NSC differentiation into neurons. In the case of ER which decrease the proliferation of NSCs, the activated ER may cause the down-regulation of PPARγ expression (33). Further investigations are needed to illustrate the networks of complex transcriptional regulation by nuclear receptors.

In this review, we focused on NSCs because it is one of the most attractive stem cells in regenerative medicine, drug discovery, and stem cell biology research (see Fig. 3). Another promising kind of stem cell is a multipotent adult progenitor cell (MAPC) that is obtained from bone marrow and other parts of the body. MAPCs differentiate at the single cell level into most mesodermal cell types as well as cells with neuroectodermal and endodermal features in vitro (34, 35). Thus, MAPC may be an ideal source for therapy of inherited or degenerative diseases. Concurrently, MAPC may provide useful information for the research on other stem cell types. An interesting question, for example, is whether MAPC can transiently acquire a NSC-like phenotype during differentiation into cells with neuroectodermal characteristics in vitro, or whether TLX and PPARγ can inhibit the transdifferentiation of MAPC into neuronal lineage (see Fig. 3).

A recent challenging project in stem cell research is to find the stemness gene, key molecular switches that maintain the nature of stem cells. Based on the idea that some properties of stem cells may be universal, researchers tried to identify stem cell markers using gene

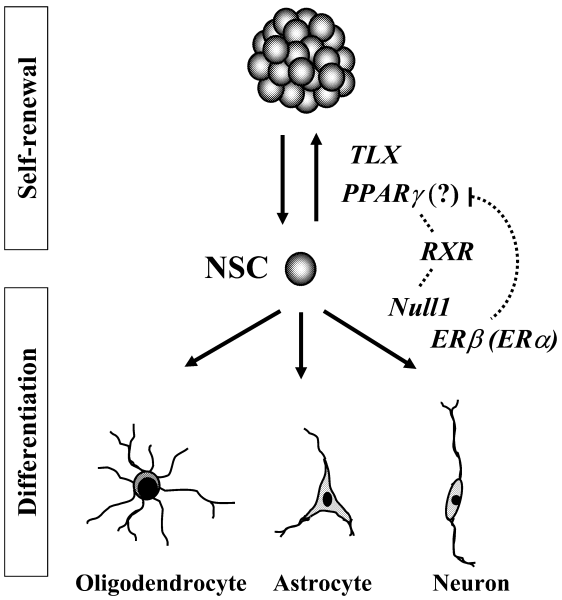

Fig. 3. Schematic illustrations of neural stem cells on self-renewal and differentiation. TLX: a mouse homolog of the *Drosophila tailless* gene, RXR: retinoid X receptors.

chip technology to search for a common signal among different kinds of stem cells, a genetic profile that would in essence define the nature of stemness (36, 37). Ivanova and colleagues found 283 genes (including eight nuclear receptor subfamily and some nuclear receptor coactivators) that were expressed in all three different stem cells: hematopoietic, embryonic stem (ES) cells, and NSCs (36). Douglas Melton's laboratory also reported that a total of 216 genes are enriched in all three of their stem cells (37). The two sets of genes, however, were almost completely different, sharing only six genes. Additionally, another group found 385 genes in similar experiments with retinal stem cells, ES cells, and NSCs, but identified only one gene (integrin $\alpha 6$) in the three individual studies (38). Although there is a possibility that the failure to identify stemness genes was due to the technical difficulties of the experiments, such as isolation of stem cells or the resolution power of the gene chip technology, these data may suggest that different gene networks to retain self-renewal capacity or multipotency are used in the different stem cell types. Although stem cell research has been advanced in the last few decades, the mechanisms involved in self-renew and differentiation of stem cells remains unclear. We believe that clarifying the role of nuclear receptors in stem cells may also be of great interest.

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