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Chapter 3

A Critical Risk Factor for a Major Side Effect of Interferon-Alpha Therapy: Activated Indoleamine 2,3-Dioxygenase 1 is Related to Depressive Symptoms

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.71013

Abstract

Hepatitis C virus (HCV) infection affects approximately 170 million people worldwide. Interferon-alpha (IFN-α) is a cytokine that is related to early viral infection and has both antiviral and antiproliferative properties. The current standard treatment for long-term chronic hepatitis C (CHC) consists of combination therapy with IFN-α and ribavirin, which has a broad spectrum antiviral effect. Despite the potential therapeutic benefits of IFN-α, its administration often causes many side effects, such as somatic and neuropsychiatric symptoms. Depression is a serious and frequently occurring side effect of IFN-α therapy, and this is one of the major reasons for cessation of the therapy. Therefore, in order to avoid the discontinuation of INF-α therapy owing to depressive symptoms, it is important to identify the risk factor(s) leading to the onset of associated depressive symptoms. In this chapter, we introduce our novel findings on the association between IFN treatment and the onset of depression in CHC patients as well as the potential neurobiological mechanisms by which depression may arise. We also highlight a potential approach for predicting the onset risk of depression as a side effect in these patients.

Keywords: hepatitis C, IFNs, depression, tryptophan catabolism, indoleamine 2,3-dioxygenase 1

1. Introduction

Hepatitis C virus (HCV) infection is a global health problem. Up to 85% of HCV-infected patients may develop long-term chronic hepatitis C (CHC), a disease state associated with serious clinical sequela, including liver cirrhosis, hepatic fibrosis, and hepatocellular carcinoma [1–4]. It has been estimated that up to 20% of CHC patients will develop hepatic cirrhosis over

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a 20–25-year period, and these individuals are at an increased risk for developing end-stage hepatic diseases or hepatocellular carcinoma [4]. Therefore, aggressive antiviral treatments to successfully induce viral remission constitute a major strategy for reducing the morbidity and mortality associated with CHC.

Immunotherapy with interferon-alpha (IFN-α) is commonly used to treat CHC and several types of malignancies owing to its antiviral, antiproliferative, and immunoregulatory effects [5]. In clinical trials, more than 50% of CHC patients treated with combination therapy using IFN-α and ribavirin achieved a sustained viral response, defined as undetectable HCV in the blood 6 months following the end of treatment [4]. Despite the efficacy of IFN-α in CHC treatment, IFN-α therapy causes serious side effects; early signs include somatic symptoms (anorexia, pain, insomnia, fever, and fatigue). Prolonged therapy causes neuropsychiatric symptoms including depressive states, anhedonia, anxiety, and cognitive impairment. In particular, depression is a serious and frequently occurring side effect of IFN-α therapy, and this leads to discontinuation of the therapy in up to 45% of patients [6, 7]. Therefore, in order to avoid the discontinuation of IFN-α therapy owing to depressive symptoms induced by the cytokine, it is important to identify the risk factor(s) leading to the associated depressive symptoms.

A number of findings suggest that the neuropsychiatric side effects observed during IFN-α therapy may be linked to aberrations in the tryptophan (TRP)-kynurenine (KYN) pathway [8, 9]. Clinical studies have found that IFN-α therapy reduces plasma TRP and serotonin (5-hydroxythrptamine; 5-HT) levels [8] and increases KYN levels in plasma and cerebrospinal fluid (CSF). In addition, the KYN/TRP ratio, an index of indoleamine 2,3-dioxygenase 1 (IDO1) activity, is increased in patients receiving IFN-α therapy [8].

IDO1 is an extrahepatic enzyme that catalyzes the conversion of TRP to KYN, which can produce many neuroactive metabolites such as 3-hydroxykynurenine (3-HK), kynurenic acid (KA), and quinolinic acid (QUIN). Intriguingly, QUIN levels in CSF have been found to correlate with the severity of depressive pathology [10], and post-mortem studies have shown increased microglia QUIN levels in the frontal cortex of severely depressed patients [11].

In the current chapter, we present the findings of our latest study, which demonstrates the association between IFN treatment and changes in the TRP-KYN pathway in the blood of HCV patients. To do so, we investigated the effect of chronic *Ifn* gene expression on depression-like behavior and levels of brain TRP-KYN metabolites in mice. Our results suggest the possibility for the prediction of onset risk of depression as a side effect in HCV patients.

2. Molecular characteristics and antiviral mechanisms of interferons (IFNs)

2.1. Classification of IFNs

IFNs were first introduced in 1957 as antiviral molecules. Based on their receptor types on the cell membrane surface, IFNs are classified into type I and type II. IFN type I mainly consists of IFN-α/β, while IFN type II consists of IFN-γ. IFN type I is a family of cytokines in which their

http://dx.doi.org/10.5772/intechopen.71013

amino acid sequence similarity reaches 30–80%. They are produced by a wide variety of cells, including fibroblasts, epithelial cells, and hepatocytes [12, 13]. However, in most viral infections, plasmacytoid dendritic cells (pDCs) are probably the major source of these cytokines. In contrast, IFN type II (IFN-γ) is a single gene cytokine unrelated in structure to IFN-α/β, which is produced largely by macrophages, natural killer (NK) cells, and T lymphocytes [12].

2.2. Immune response for HCV infection and IFN induction

The host response against HCV infection is first triggered when a pathogen-associated molecular pattern (PAMP), presented by an infecting virus, is recognized and engaged by specific PAMP receptors expressed on the host cells. This leads to the activation of signals that ultimately induce the expression of antiviral effector genes [14, 15] (**Figure 1**). For RNA viruses, protein, and nucleic acid products of infection or replication have been identified as viral PAMPs. These are engaged by specific toll-like receptors (TLRs) or nucleic acid-binding proteins that serve as PAMP receptors [15–17]. The viral RNA of HCV contains each of these PAMP signatures, and is adequate to trigger the host response when introduced into naïve cells [18, 19]. In hepatocytes, which is the target cell of HCV infection, independent pathways

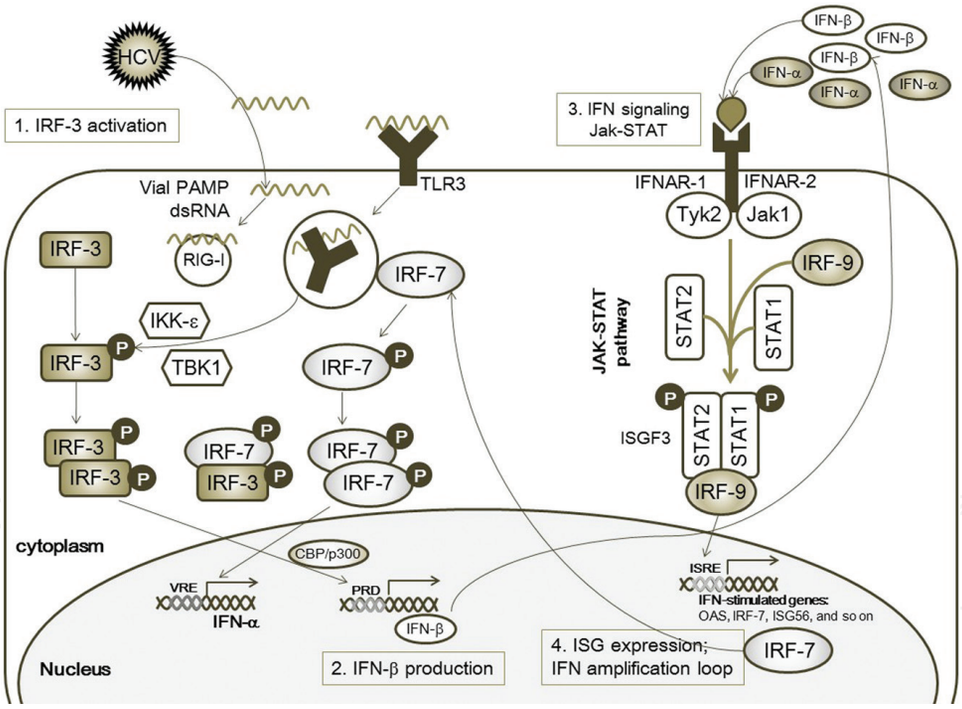

Figure 1. The host innate response to HCV infection. Adapted from Ref. [14]. (1) HCV RNA binding to RIG-I or TLR3 results in the activation IRF-3. The dimer of phospho-IRF-3 translocates to the nucleus, interacts with transcription partners and binds to the cognate-DNA PRD in the promoter region of IRF-3 target genes. (2) IRF-3 activation leads to the induction of IFN-β production. (3) Secreted IFN-β from the infected cells binds to the IFN-α/β receptor, and results in activation of the JAK-STAT pathway. The ISGF3 complex translocates to the nucleus, where it binds to the ISRE on target genes to direct ISG expression. IRF-7 is one of the ISGs and it is activated after expression through viral PAMP signaling. (4) The IRF-7 dimer and heterodimer with IRF-3 binds to VRE in the promotor region of IFN-α genes resulting in the production of various IFN-α subtypes and establishing a positive-feedback loop for IFN amplification. It is the IFN-α component of the host response that is exploited by the current IFN-based therapy for HCV infection [14].

of retinoic acid-inducible gene I (RIG-I) and TLR3 signaling construct two major pathways of host defense triggered by double-stranded (ds) RNA [19–21]. Viral PAMP binding to RIG-I or TLR3 results in the phosphorylation and activation of interferon regulatory factor 3 (IRF-3) by TANK-binding kinase 1 (TBK-1) and I kappa B kinase ε (IKK-ε) [14, 22]. The dimer of phospho-IRF-3 translocates to the cell nucleus, interacts with its transcription partners, including CREB-binding protein (CBP)/p300, and binds to the cognate-DNA positive regulatory domain (PRD) in the promoter region of IRF-3 target genes, such as IFN-β [14, 23]. The engagement of PAMP receptors also leads to the synthesis of IFN-α/β, tumor necrosis factor (TNF), and a variety of other cytokines, which are largely produced by mainly pDCs that express TLRs in abundance. IFN-α/β produced by pDCs activates NK cells, thereby enhancing their cytotoxic potential and stimulating their production of IFN-γ. IFN-α/β produced by pDCs also modulates the activation of $CD8^+$ T cells, which produce additional IFN-γ and represent the central players in the pathogen-specific adaptive immune response [12].

2.3. The antiviral effect of IFNs on HCV

IFN-α mediates a wide range of biological activities including antiproliferation, immunomodulation, and antiviral responses. IFN-α/β acts to induce the antiviral response in cells. These cells can be far from IFN-α/β production site and IFN-α/β interacts with specific cell surface receptors, type I IFN receptors (interferon-alpha receptor 1 (IFNAR1) and IFNAR2; **Figure 1**). IFNARs signal to the nucleus via Janus kinase-1 (Jak1) and tyrosine kinase 2 (Tyk2) phosphorylation of the signal transducers and activators of transcription (STATs) [24]. The classic IFN-α/β signaling pathways activate STAT1/STAT2 heterodimers and the trimeric IFN-stimulated gene factor (ISGF) complex containing IRF-9, which activate the expression of specific subsets of genes controlled by promoters containing interferon-stimulated response elements (ISRE; **Figure 1**) [15]. Interferon-stimulated genes (ISGs) are the genetic effectors of the host response, although the details of the signaling mechanisms by which IFN-α/β and IFN-γ induce the transcription of ISGs are still being defined [25]. IRF-7 is a transcription factor and an ISG. It is expressed in many tissue types, including complex liver tissue, in response to IFN. IRF-7 is activated after expression via viral PAMP signaling pathways that overlie with the IRF-3 activation pathway. IRF-7 phosphorylation, dimerization, and heterodimerization with IRF-3 lead to bind its cognate virus-responsive element (VRE) in the promotor region of IFN-α genes. Then, this binding results in the production of various IFN-α subtypes. The transcription effector action of IRF-7 also promotes diversification of the ISG response, establishing a positive-feedback loop that amplifies IFN production, and antiviral action [14]. This increases the plenty of RIG-I and viral PAMP signaling modules whose continued signaling acts to amplify IFN production and the host response. The medicinal administration of IFN-α promotes an antiviral reaction against HCV infection by stimulating ISG expression via the IFN-α/β receptor and the JAK-STAT pathway. In addition to stimulating ISG expression, IFN-α induces or promotes the maturation of immune effector cells, and enhances the production of other cytokines by resident hepatic cells to indirectly modulate the cell-mediated defenses and adaptive immunity to HCV [15]. Viral trigger and control of the host response may elucidate cellular tolerance for HCV RNA replication and influence the outcome of infection.

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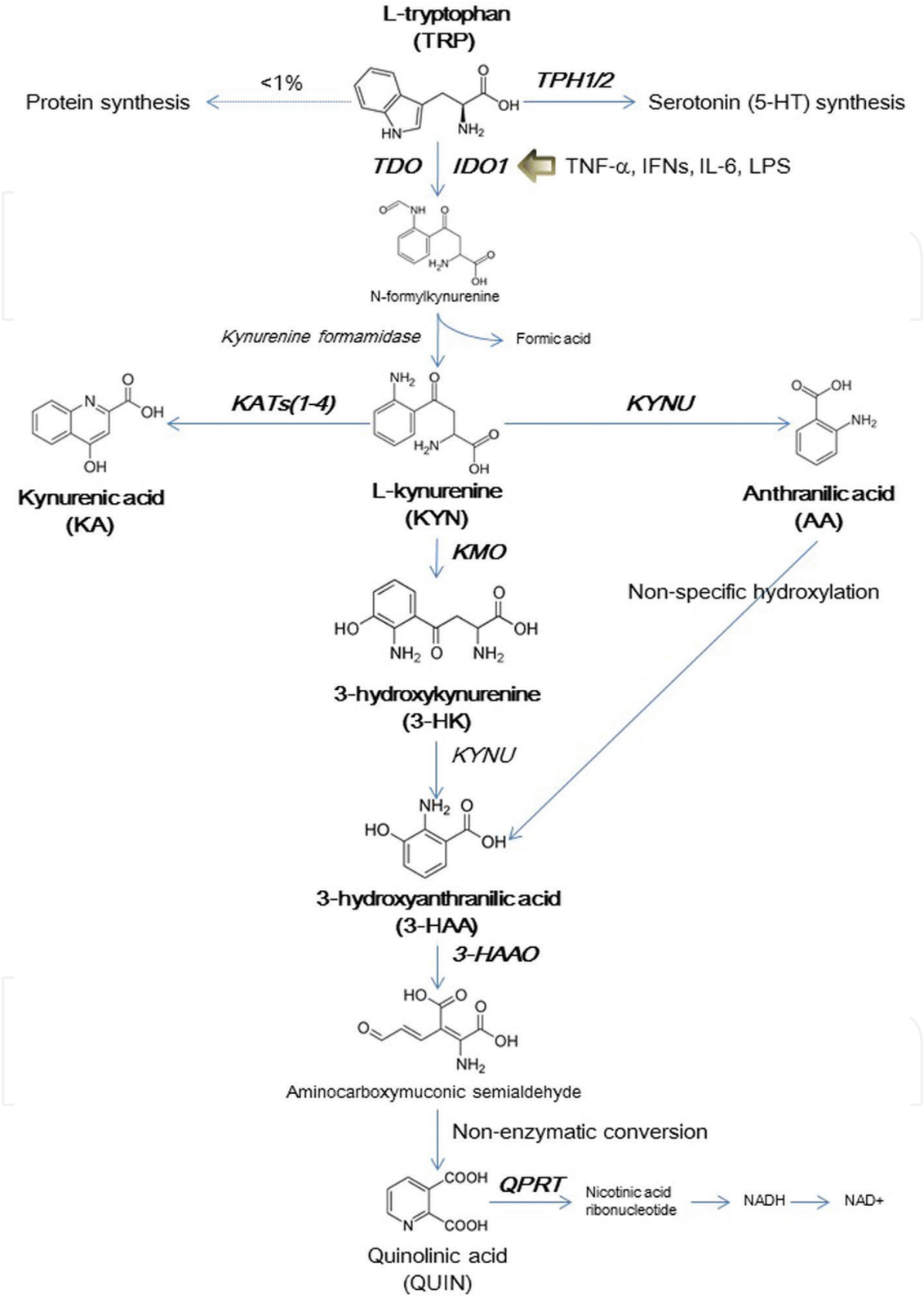

Figure 2. Schematic overview of the TRP-KYN pathway. IDO1 catabolizes L-TRP to N-formyl-L-kynurenine, which is converted to L-KYN by formamidase. L-KYN is further metabolized to AA by kynureninase (KYNU), to KA by kynurenine aminotransferases (KATs), and to 3-HK by kynurenine 3-monooxygenase (KMO). KMO is then metabolized to 3-HAA by 3-hydroxyanhranilate 3,4-dioxygenase (3-HAAO). 3-HAA is further metabolized to QUIN.

3. Side effect: IFN-induced depression and tryptophan metabolism

IFN-α has been shown to develop depression in many diseases, not only CHC, but also in melanoma, chronic myelogenous leukemia, and renal cell carcinoma [26]. However, CHC patients may be more susceptible to developing IFN-induced depression than patients with other disorders, possibly due to a baseline 5-HT system dysfunction. Depression in CHC patients may result from changes in platelet 5-HT function, with decreased 5-HT concentrations during CHC infection compensated for by a decrease in reuptake and metabolism [1]. Immune activation, particularly by IFN-γ, affects the catabolism of TRP, a precursor of 5-HT, by inducing expression of IDO1. IDO1 is the first and rate-limiting enzyme that converts TRP to N-formyl-L-kynurenine, which is further metabolized to QUIN (**Figure 2**). IFN treatment of CHC patients results in a decrease in plasma TRP and an increase in plasma KYN [8]. Another clinical study with cancer patients has shown that immunotherapy with IFN-α significantly increases the severity of depressive symptoms, which is related to a depletion of serum 5-HT and induction of the catabolism of TRP to KYN [27]. Thus, TRP catabolism switches from the 5-HT pathway to the KYN pathway, resulting in a decrease in 5-HT levels.

IDO1 is induced by several pro-inflammatory cytokines including IFNs (IFN-α/β, γ), TNF-α, and interleukin 6 (IL-6). It is also widely accepted that IFNs, especially IFN-γ, are essential factors for IDO1 induction since two ISREs and IFN-γ-activated site (GAS) element sequences are found in the 5′-flanking region of the IDO1 gene [28]. Recent preclinical studies in mice have demonstrated that pharmacological inhibition of IDO1 enzymatic activity or genetic deletion of IDO1 abrogates acute and chronic inflammation-dependent behavioral changes induced by peripheral or central administration of lipopolysaccharide (LPS) [29–33]. Additionally, it has been reported that peripheral administration of KYN alone can induce depression-like behavior in rats [34]. In a clinical study, patients receiving IFN-α therapy showed increases in the total Montgomery-Asberg Depression Rating Score (MADRS), an index of depressive symptoms similar to the KYN/TRP ratio; this indicates IDO1 activity and the KYN/KA ratio, which reflects a neurotoxic challenge [35]. These findings suggest that only TRP depletion itself may not be required for the induction of behavioral changes as a result of IDO1 activation; and that KYN and its neuroactive metabolites are more related to cytokine-induced depression-like behaviors than TRP depletion. However, it is still unclear whether direct activation of IDO1 and KYN metabolites plays a definitive role in the induction of depressive symptoms by IFN-α treatment.

4. The association between IFN treatment and changes in the TRP-KYN pathway on depression as a side effect in humans and mice

4.1. Changes in the levels of serum TRP and its metabolites in HCV patients with IFN-α therapy

In order to further clarify the relationship between the IDO1-induced KYN pathway and the development of depressive symptoms during IFN-α therapy, we conducted a study in which we measured TRP metabolites of the KYN pathway in the serum of HCV patients undergoing IFN-α therapy.

A total of 49 patients (32 males and 17 females; mean age 54.0 ± 2.3 years) suffering from CHC were recruited. **Table 1** shows the clinical characteristics of patients with HCV. In this study, most of patients were treated with recombinant (r) IFN-α 2b or pegylated (PEG)-IFN-α 2b (21 patients (42.9%) received each medicine, respectively). Five patients (10.2%) were treated with natural (n) IFN-α, and others received PEG-IFN-α 2a (2.0%) and rIFN-α 2a (2.0%), individually. All interferons have almost the same efficiency and induce about the same activation of the KYN pathway [36]. No patient had a past record of psychiatric treatment, and all were off from depressive symptoms prior to IFN-α treatment. They did not take any antidepressant medications during the study period. At an average of 104.2 ± 15.8 days after the IFN-α administration, some patients presented with apathy, social isolation tendencies, melancholy, depressed mood, and an intention to stop IFN administration. Patients who felt depressed mood were referred for psychiatric

(a) Clinical characteristics of HCV patients

	Depression (–)	Depression (+)
All subjects	30 (male: 20; female:10)	19 (male: 12; female: 7)
Age	54.33 ± 2.06	54.0 ± 2.29
HCV genotype 1b	24 (80%)	15 (78.9%)
HCV genotype 2a	4 (13.3%)	3 (15.8%)
HCV genotype 2b	2 (6.7%)	1 (5.3%)
AST	59.43 ± 5.09	57.47 ± 6.45
ALT	82.68 ± 11.36	69.56 ± 8.65

"Depression (−)": HCV patients without depression, "Depression (+)": HCV patients with depression following IFN-α therapy [47]. HCV, hepatitis C virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

(b) The time points of blood sampling

Time points	Depression (–) (mean ± SEM)	Depression (+) (mean ± SEM)	*t*	df	p value
(a) Before the onset of therapy	1–35 d (6.3 ± 1.8 d)	0–22 d (6.7 ± 1.3 d)	0.230	48	0.819
(b) 2 w after the onset of therapy	13–15 d (13.8 ± 0.1 d)	12–15 d (13.6 ± 0.2 d)	0.513	61	0.610
(c) 4 w after the onset of therapy	25–30 d (27.9 ± 0.1 d)	25–29 d (27.6 ± 0.3 d)	0.952	40	0.347
(d) The period of therapy	167–343 d (252.0 ± 15.7 d)	54–337 d (183.4 ± 22.0 d*)	2.592	46	0.013

For all HCV patients, blood was collected before the onset of IFN-α therapy, as well as 2 and 4 weeks after the onset of therapy, and after the end or cessation of therapy. See **Figure 3a** for a detailed blood sampling schedule.*$p<0.05$ *versus* Depression (−) [47].

Table 1. Clinical information for HCV patients undergoing IFN-α therapy.

evaluation and identified as major depressive disorder (MDD) by a psychiatrist. Nineteen of the HCV patients were diagnosed with depressive symptoms [depression (+)], while 30 of them did not present depressive symptoms [depression (−)]. The diagnosis to verify the incidence of depressive symptoms associated to MDD was made according to the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders fourth edition) and ICD-10 (International Statistical Classification of Disease and Related Health problems-10) base on clinical interviews.

For all HCV patients, blood was collected before the onset of IFN-α therapy as well as 2 and 4 weeks after initiation of treatment. There was a no significant time difference for blood sampling between depression (−) and (+) patients (**Table 1b** and **Figure 3**).

Previous studies suggested that IDO1-mediated TRP metabolism could be implicated in the development of depression, as a side effect of IFN-α therapy in HCV patients. We also found that HCV patients showed decreased TRP and increased KYN concentrations without any changes in KA, AA, and 3-HAA concentrations during IFN-α therapy (**Figure 3b** and **Table 2a**). Furthermore, depression (+) patients presented a higher increase in 3-HK concentration compared to depression (−) patients during treatment (**Table 2a**). Ogawa et al. recently showed that plasma TRP concentration was significantly decreased in MDD patients compared to healthy controls [37]. Teraishi et al. also demonstrated increased KYN metabolites along the TRP-KYN-QUIN pathway, but not the KYN-KA pathway, in MDD patients [38]. Our results showed that the level of 3-HK in the serum significantly increased in depression (+) patients are consistent with these findings. We also investigated the ratios of 3-HK/KA (reflecting neurotoxic indices) [39, 40] and KYN/TRP (reflecting IDO1 activity) in depression (−) and depression (+) HCV patients during IFN-α treatment (**Figure 3c** and **Table 2b**). The ratios of KYN/TRP and 3-HK/KA in both groups increased during treatment. However, in depression (+) patients, the ratios of KYN/TRP and 3-HK/KA increased much larger in depression (−) patients during treatment (**Table 2b**). In these patients, the serum KYN/TRP and 3-HK/KA ratios increased more at the diagnosis of depression, but at 70.3 ± 9.1 days post therapy, they returned to the same levels as before onset of the therapy (data not shown). The severity of depression was not assessed during treatment, using neither the MADRS nor Hamilton Depressing Rating Scale. Therefore, we could not clearly show the direct association between the aggravation of depressive symptoms and changes in TRP metabolites. However, our results suggest that HCV patients with a high sensitivity for IDO1 activation by IFNs are highly susceptible to the depression-related side effects of IFN-α treatment.

4.2. The effects of chronic *Ifn-γ* gene expression on depression-like behavior in mice

We hypothesized that the high induction of IDO1 and the imbalance of TRP metabolites induced by IFNs in humans may be related to psychiatric side effects, such as depression. Previous studies have shown that all three IFNs (IFN-α, -β, and -γ) induce strong IDO1 activity in human peripheral blood mononuclear cells [41, 42]. In contrast, in mouse, IDO1 is induced more markedly by IFN-γ than IFN-α, which has only a weak direct IDO1-stimulatory effect. Therefore, we investigated whether IDO1 activity induced by *Ifn-γ* gene transfer impaired behavior in mice.

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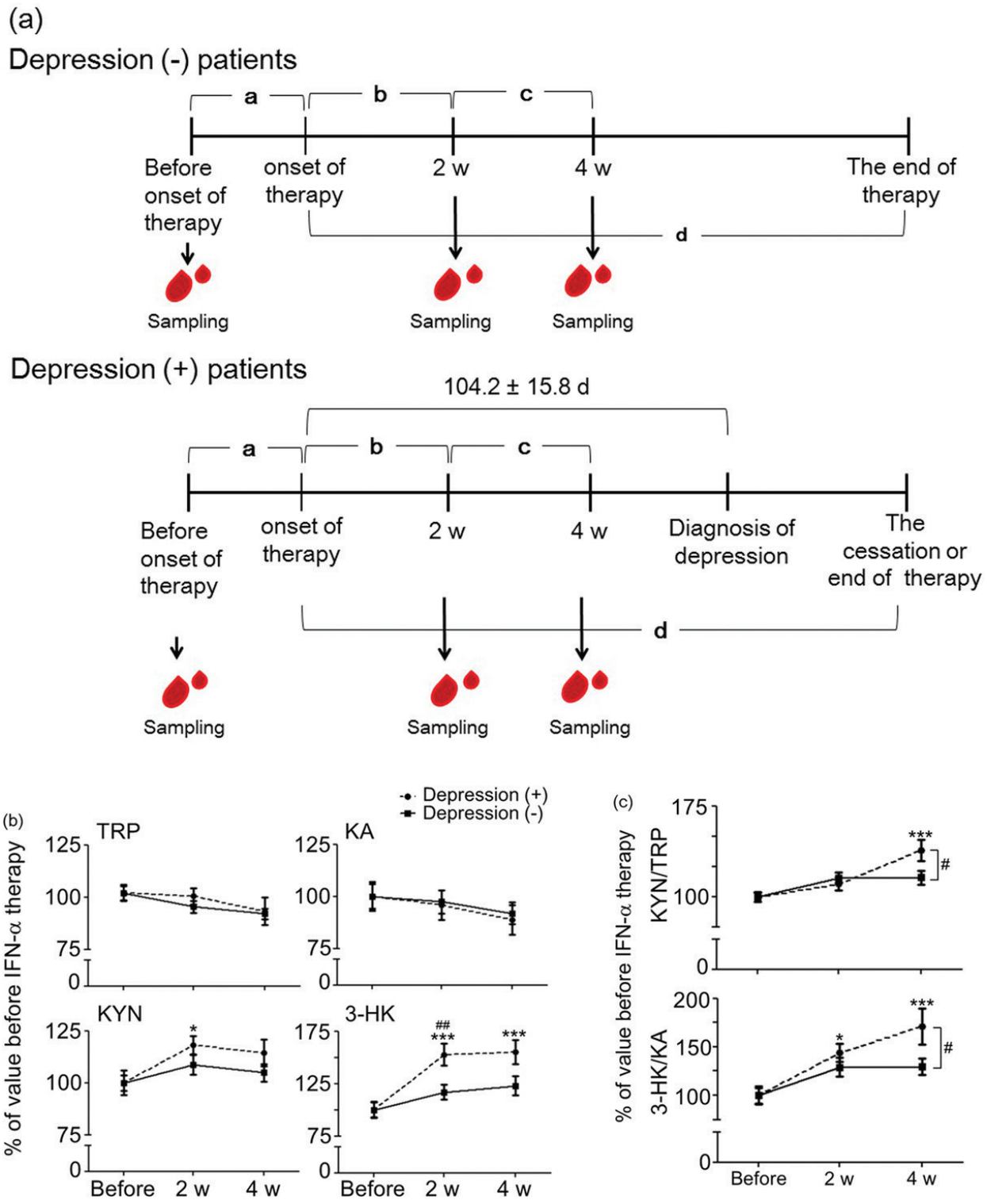

Figure 3. Changes in the levels of serum TRP and its metabolites in HCV patients receiving IFN-α therapy. Original data from Ref. [47]. (a) Schematic depiction of the collection schedule for blood sampling from depression (−) and depression (+) HCV patients. The range of time points and average collection time point (a–d) per group are listed in **Table 1b**. (b) Serum TRP, KYN, KA, and 3-HK concentrations in HCV patients at 2 and 4 weeks after the onset of therapy, expressed as a percentage of the concentration before IFN-α therapy. (c) Serum KYN/TRP and 3-HK/KA ratios in HCV patients are shown as a percentage of values before IFN-α therapy. Rectangles indicate non-depressive HCV patients [Depression (−)] and circles indicate HCV patients with depressive symptoms [Depression (+)]. Each data point represents the mean ± SEM of values obtained from n = 30 depression (−) patients and n = 19 depression (+) patients. $^{*}p<0.05$, $^{***}p<0.001$ *versus* before the onset of IFN-α therapy, $^{\#}p<0.05$, $^{\#\#}p<0.01$ *versus* depression (−) patients. Detailed statistical analyses are shown in **Table 2** [47].

(a) Changes in the levels of serum TRP and its metabolites					
	% of value before IFN-α therapy		*t*	df	*p* value
	Depression (–)	Depression (+)			
2 w after onset of therapy					
TRP	95.4 ± 2.93	100.5 ± 3.98	0.965	40	0.340
KYN	108.6 ± 4.77	118.1 ± 4.24*	1.200	39	0.237
3-HK	117.0 ± 7.13	152.6 ± 10.4***, ##	2.886	38	0.006
KA	97.4 ± 5.51	95.9 ± 7.13	0.136	38	0.892
AA	119.9 ± 7.42	115.5 ± 7.11	0.381	41	0.706
3-HAA	102.9 ± 6.53	121.8 ± 12.6	1.452	37	0.155
4 w after onset of therapy					
TRP	92.0 ± 2.55	93.3 ± 6.49	0.213	39	0.833
KYN	104.8 ± 4.38	114.4 ± 6.38	1.204	39	0.236
3-HK	123.0 ± 9.01	155.0 ± 11.5***	2.005	36	0.053
KA	91.9 ± 5.12	88.8 ± 6.98	0.341	40	0.735
AA	107.5 ± 5.32	103.6 ± 11.3	0.361	40	0.720
3-HAA	101.9 ± 6.52	104.5 ± 14.8	0.182	36	0.857

Percent value of serum TRP, KYN, 3-HK, KA, AA, and 3-HAA concentrations in HCV patients at 2 and 4 weeks after the onset of therapy, compared to the concentration (100%) before IFN-α therapy. In the clinical samples, some metabolites were difficult to separate clearly by HPLC. Therefore, the degree of freedom (df) values differ by the measured molecules. "Depression (–)": HCV patients without depression, "Depression (+)": HCV patients with depression.*$p<0.05$,

***$p<0.001$ *versus* before the therapy;

##$p<0.01$ *versus* Depression (–) [47].

(b) Changes in serum KYN/TRP and 3-HK/KA ratios					
	% of value before IFN-α therapy		*t*	df	*p* value
	Depression (–)	Depression (+)			
2 w after onset of therapy					
KYN/TRP	115.6 ± 4.55	114.1 ± 5.95	0.198	42	0.844
3-HK/KA	129.1 ± 9.52	144.0±9.06*	1.036	39	0.308
4 w after onset of therapy					
KYN/TRP	115.7 ± 5.69	138.3±8.84*,#	2.094	35	0.044
3-HK/KA	129.6 ± 8.67	171.1 ± 18.6***,#	2.325	35	0.026

Serum KYN/TRP reflects IDO1 activity, and 3-HK/KA reflects neurotoxic indices. Both ratios in HCV patients were shown as % of value compared to the value (100%) before IFN-α therapy, at 2 and 4 weeks after the onset of therapy.*$p<0.05$,

***$p<0.001$ *versus* before the therapy;

#$p<0.01$ *versus* Depression (–) [47].

Table 2. Changes in TRP-KYN pathway in HCV patients undergoing IFN-α therapy.

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To conduct this experiment, for murine *Ifn-γ* gene transfer, the plasmid pCpG-Muγ was constructed by inserting a BglII/NheI murine *Ifn-γ* cDNA fragment into the BglII/NheI site of the pCpG-mcs vector (**Figure 4a**). The prepared plasmid pCpG-Muγ was dissolved in normal saline and injected into the tail veins of the mice for over 5 s on day 0. The injection volume was approximately 9% (v/w) of body weight. To eliminate the possibility of tissue damage or inflammation by the hydrodynamic injection, a control plasmid, which was the empty vector without the *Ifn-γ* gene (pCpG-mcs), was injected (0.05 pmol/mouse; IFN-γ transfected (−) mice). A previous study demonstrated that sustained IFN-γ concentrations were observed in mice receiving pCpG-Muγ at a dose of 0.2 pmol/mouse and more than 1000 pg/mL of IFN-γ was detected in the serum from 6 to 31 days after injection of pCpG-Muγ [43]. We also confirmed that the injected plasmid, pCpG-Muγ (IFN-γ transfected (+) mice) significantly increased IDO1 activity in the frontal cortex over a dose of 0.05 pmol/mouse compared to IFN-γ transfected (−) mice (**Figure 4c**). Therefore, the plasmid dose was fixed at 0.05 pmol/mouse for subsequent experiments, which corresponded to 0.10–0.12 μg of DNA/mouse.

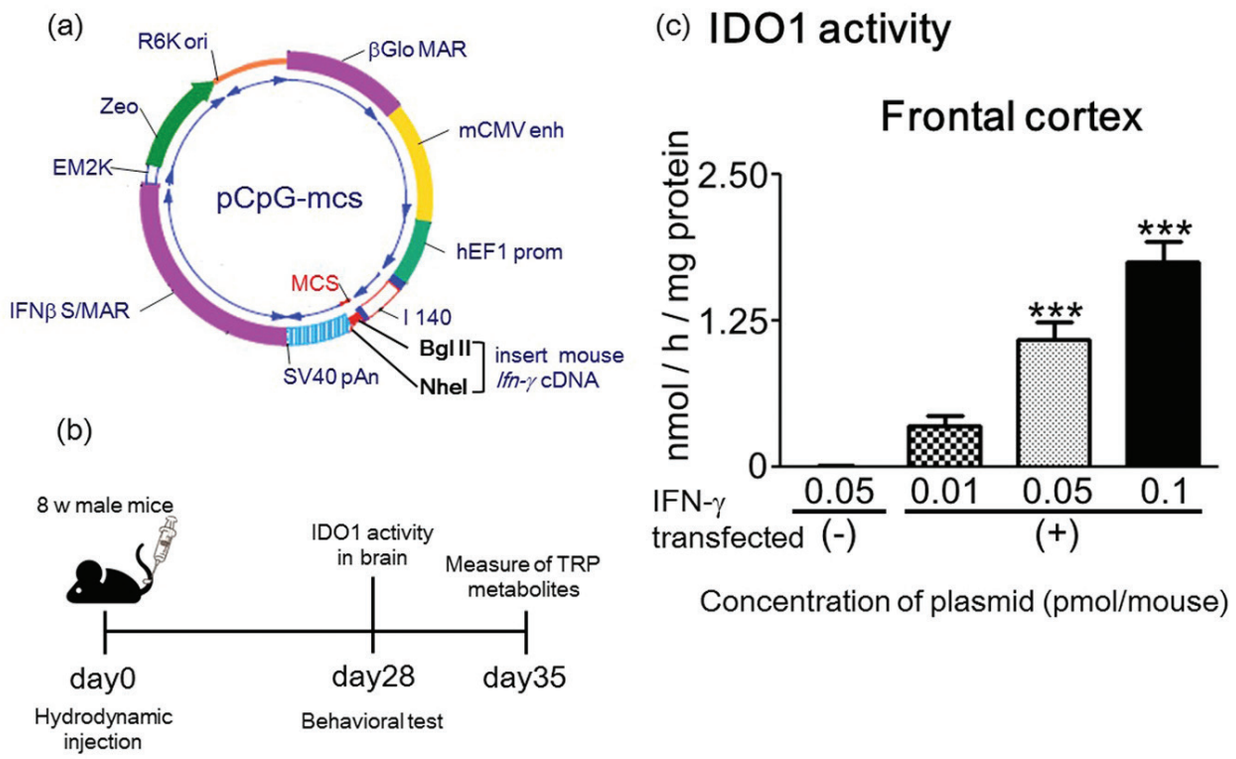

Figure 4. *Ifn-γ* gene transfer. Original data from Ref. [47]. (a) Schematic depiction of the pCpG-Muγ plasmid construct (InvivoGen, San Diego, CA). (b) Schematic depiction of the time schedule for animal experiments. (c) Increase of IDO1 activity in the frontal cortex of mice 28 days after *Ifn-γ* gene transfer [47]. βGlo MAR, β-globin matrix attachment region; mCMV enh, mouse cytomegalovirus enhancer; hEF1 prom, human elongation factor1 promoter; I140, synthetic 5′UTR containing an intron 140; MCS, multi cloning site; SV40 pAn, Simianvirus 40 polyadenylation; IFN-β S/MAR, interferon β gene scaffold/matrix attachment region; EM2K, CpG-free version of the bacterial EM7 promoter; Zeo, Zeocin; R6K ori, R6K origin.

In order to clarify whether the activation of IDO1 by IFN-γ-affected behaviors, three tests, open-field test (OFT), the Y-maze test, and forced swimming test (FST), were performed in mice. Mice were transfected with either a pCpG-mcs plasmid (control vector) that did not contain the *Ifn-γ* gene [IFN-γ-transfected (−) mice] or a pCpG-Muγ plasmid that long-lasting expressed *Ifn-γ* [IFN-γ transfected (+) mice]. No significant differences in locomotor activity of the OFT was observed between IFN-γ transfected (−) and (+) mice. Similarly, in the Y-maze test, no significant differences in the alternation behavior were detected between the two groups of mice. However, in the FST, immobility time was significantly longer in IFN-γ-transfected (+) mice (**Figure 5a**). Our findings strongly suggest that IDO1 induction by IFN-γ is a critical factor in depression-like behaviors but not in short-term memory or locomotor activity in mice.

4.3. Changes in the levels of TRP and its metabolites in the serum and frontal cortex of mice following chronic Ifn-γ gene expression

In order to further elucidate the relationship between the IDO1-induced KYN pathway and the development of depression-like behavior in mice transfected with the pCpG-Muγ plasmid, we measured TRP metabolites in the serum and frontal cortex of these mice.

The serum and the frontal cortex were corrected from mice immediately following behavioral testing to determine the levels of TRP, KYN, KA, 3-HK, 3-HAA, and AA (**Figure 5b** and **c**). The concentration of serum TRP was significantly decreased in IFN-γ transfected (+) mice compared to IFN-γ-transfected (−) mice. In contrast, the levels of serum KYN and 3-HK were significantly increased in the IFN-γ-transfected (+) mice (**Figure 5b**). In the frontal cortex, IFN-γ transfected (+) mice had significantly higher KYN and 3-HK levels than the IFN-γ-transfected (−) mice. The TRP and KA levels in the frontal cortex tended to be lower in the IFN-γ-transfected (+) mice (**Figure 5c**). The activation of IDO1 by *Ifn-γ* gene transfer significantly modified the levels of TRP and its metabolites not only in the serum, but also in the frontal cortex of mice. These results suggest that an alternative explanation for the participation of IDO1 in IFN-γ-induced depression-like behavior is the generation of neuroactive TRP metabolites. This interpretation is consistent with our clinical data and previous studies by O'Connor et al. and Wichers et al. [32, 35].

4.4. The effects of Ido1 gene-deficiency on depression-like behavior, changes in TRP metabolism, 5-HT, and its turnover in the frontal cortex of mice following chronic Ifn-γ gene expression

Additionally, we evaluated the role of IDO1 in the development of depression-like behavior after *Ifn-γ* gene transfer using *Ido1* gene knockout (KO) mice, and determined the levels of TRP metabolites in the frontal cortex.

The increase in time spent in an immobile posture in the *Ifn-γ*-transfected (+)/wild type mice was significantly improved in *Ido1* KO mice (**Figure 6a**). In wild type mice, *Ifn-γ* gene transfer significantly increased the concentrations of KYN and 3-HK in the frontal cortex by 4.7- and 2.5-fold, respectively. In contrast, *Ido1* KO mice withdrew these changes in *Ifn-γ* gene transfer

http://dx.doi.org/10.5772/intechopen.71013

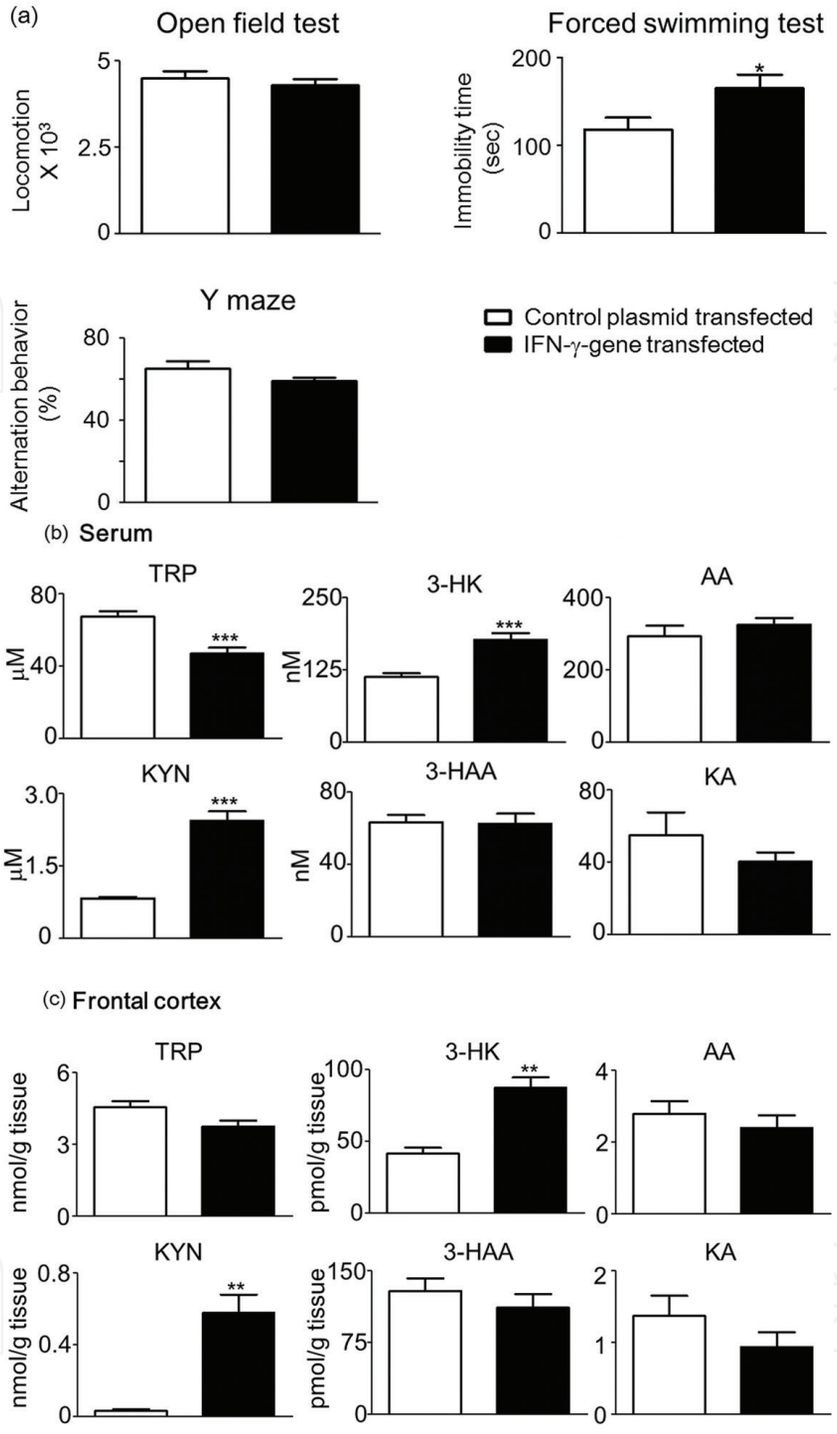

Figure 5. The effect of chronic *Ifn-γ* gene expression on the TRP-KYN pathway and depression-like behavior in mice. Original data from Ref. [47]. (a) Behavioral changes in mice 28 days after *Ifn-γ* gene transfer. Open field test shows locomotor activity of mice in a novel environment. Y-maze test shows short-term memory. Forced swim test shows depression-like behavior. Immobility time was significantly increased in IFN-γ-transfected (+) mice, compared to IFN-γ-transfected (−) mice. The open bar shows IFN-γ-transfected (−) mice, and the closed bar shows IFN-γ-transfected (+) mice. Each column represents the mean ± SEM (n = 9–16). **p* <0.05 *versus* IFN-γ-transfected (−) mice. (b) (c) Changes in the levels of TRP and its metabolites in the serum and frontal cortex of mice after *Ifn-γ* gene transfer. TRP-KYN metabolite concentrations were determined in the serum (b) and the frontal cortex (c) of mice 35 days after *Ifn-γ*-gene transfer. The open bar shows IFN-γ-transfected (−) mice, and the closed bar shows IFN-γ-transfected (+) mice. Each column represents the mean ± SEM (n = 15–20). ***p*<0.01, ****p*<0.001 *versus* IFN-γ-transfected (−) mice [47].

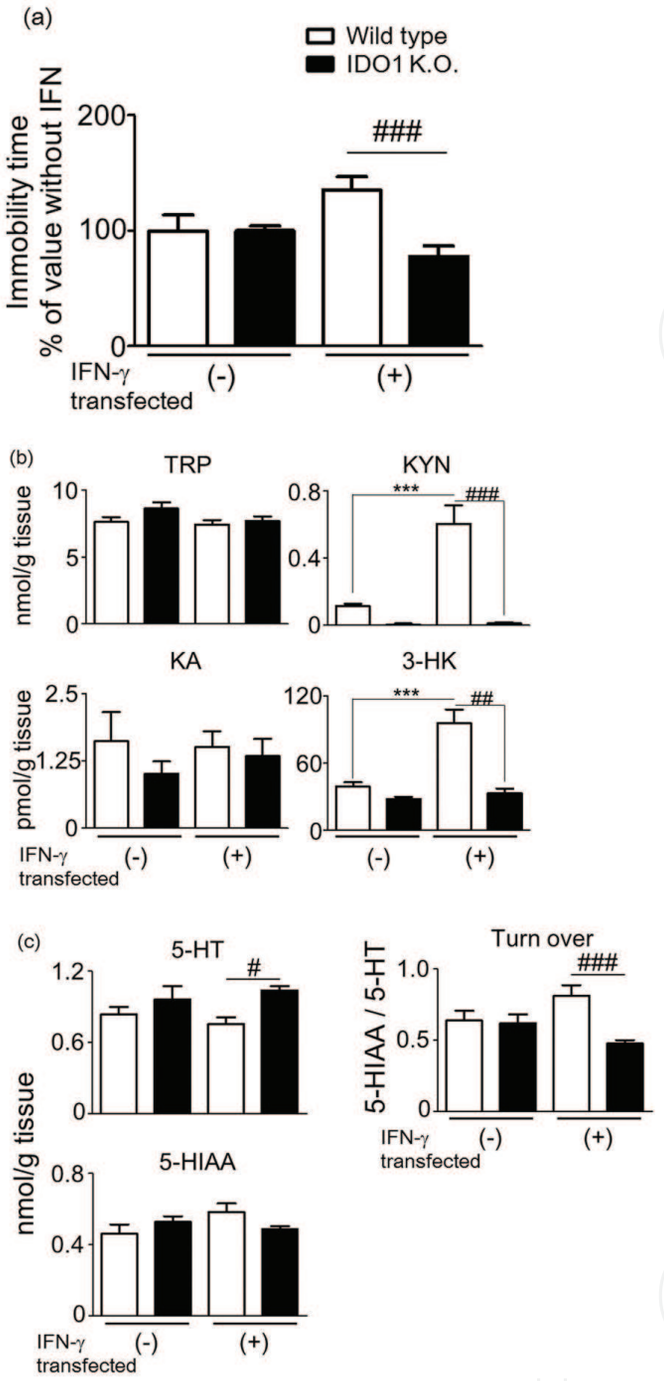

Figure 6. The effects of *Ido1* gene-deficiency on depression-like behavior, changes in TRP metabolism, 5-HT, and its turnover in the frontal cortex of mice following chronic *Ifn-γ* gene expression. Original data from Ref. [47]. (a) Abnormal behavior in a forced swimming test after *Ifn-γ* gene transfer in mice was improved in *Ido1* gene deficient mice. The Y axis shows the percent value of immobility time in IFN-γ-transfected (+) mice, compared to the time (100%) in IFN-γ-transfected (−) mice (n = 8–15). (b) The level of TRP metabolites in the frontal cortex of mice 35 days after *Ifn-γ*-gene transfer (n = 6–15). (c) The amount of 5-HT, 5-HIAA, and 5-HIAA/5-HT ratio as an index of serotonin turnover in the frontal cortex of mice 35 days after *Ifn-γ*-gene transfer (n = 6–15). The open bar represents wild type and the closed bar, *Ido1* gene deficient mice. IFN-γ-transfected (−) mice were injected with the control plasmid (pCpG-mcs), and IFN-γ-transfected (+) mice were injected with the IFN-γ-expressing pCpG-Muγ plasmid. Each column represents the mean ± SEM. *p<0.05, ***p<0.001 *versus* IFN-γ-transfected (−) wild type mice, $^{\#}p$<0.05, $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001 *versus* IFN-γ-transfected (+) wild type mice [47].

mice (**Figure 6b**). The levels of KYN and 3-HK in the frontal cortex after *Ifn-γ* gene transfer were considerably lower in *Ido1* KO mice than in wild type mice. Even though we cannot exclude the possibility that genetic deficient in *Ido1* and the resulting modifications in TRP metabolites could influence other behavioral tests, our results clearly demonstrate that *Ido1* KO mice do not show depression-like behavior and do not intensify TRP metabolites after *Ifn-γ* gene transfer.

Other studies have emphasized that the 5-HT pathway is also relevant to depression. In a clinical study, it has been shown that levels of TRP and 5-hydroxytryptophan, a precursor of 5-HT, were significantly decreased from their baseline levels in the serum of HCV patients during IFN-α therapy [44]. Thus, we speculate that biological mechanisms underlying the IFN-α treatment induced-depressive symptoms are linked not only to the activated IDO1 and KYN pathway but also to a dysfunction of the 5-HT system. To clarify on the basis of the neurotransmitter changes in depression-like behavior after *Ifn-γ* gene transfer, we measured the concentrations of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the frontal cortex of wild type and *Ido1* KO mice (**Figure 6c**). We showed that *Ifn-γ* gene transfer produced a trend toward increased 5-HIAA levels in wild type mice but not in *Ido1* KO mice. These results indicated that *Ifn-γ* gene transfer induced a potential increase in IDO1-induced 5-HT turnover. A raised 5-HT turnover suggests a process by which the availability of 5-HT to be released by neurons is decreased to compensate for neuronal dysfunction associated with depression-like behavior promoted by *Ifn-γ* gene transfer. Correspondingly, previous clinical studies have shown that brain 5-HT turnover is significantly increased in MDD patients without medication and decreased following selective serotonin reuptake inhibitors (SSRI) therapy [45, 46].

Taken together, an alternative interpretation for the involvement of IDO1 in IFN-γ-induced depression-like behavior may be that depression is related to not only the generation of neuroactive TRP metabolites but also to the alteration of serotoninergic neurotransmission.

5. Conclusion

The levels of TRP metabolites in the serum of HCV patients changed significantly. In particular, the increase in serum 3-HK concentration in depressive HCV patients was much larger than that in HCV patients without depressive symptoms. The ratios of serum KYN/TRP, reflecting IDO1 activity, and 3-HK/KA were increased in depressive and non-depressed HCV patients with therapy. However, the increase in serum KYN/TRP and 3-HK/KA ratios in depressive patients was much higher than that of non-depressive HCV patients. When the *Ifn-γ* gene was transfected into normal mice, depression-like behavior significantly increased. Additionally, *Ifn-γ* gene transfer to mice induced dramatic changes in TRP metabolite concentrations in the serum and the prefrontal cortex. On the other hand, genetic deletion of *Ido1* abrogated the enhanced depression-like behavior after *Ifn-γ* gene transfer. In conclusion, our results clearly show that IDO1 is a critical molecular regulator of the depressive pathology induced as a side effect of interferon therapy. Moreover, the depressive symptoms are induced via increases in degradation of TRP and neuroactive metabolites along the KYN

pathway, which finally changes in the alternation of 5-HT turnover. Our findings suggest that inflammatory pathways that lead to the activation of IDO1 may be a novel therapeutic target in patients suffering from inflammation-associated depression, for example, HCV or cancer therapy. Our results also suggest the monitoring of TRP-KYN metabolites during immunotherapy might assist in predicting the onset risk of depression as a side effect in these patients. However, further insight into the role of each downstream KYN pathway metabolite in the pathological process is needed to understand, and to clarify the relationship with complex neurotransmitters.

Acknowledgements

This work was supported by Grant-in Aid for Scientific Research of YM (26860368 and 16K08948).

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References

[1] Asnis GM, De La Garza R 2nd. Interferon-induced depression in chronic hepatitis C: A review of its prevalence, risk factors, biology, and treatment approaches. Journal of Clinical Gastroenterology. 2006;**40**(4):322-335. DOI: 10.1097/01.mcg.0000210099.36500.fe

[2] Hoofnagle JH. Hepatitis C: The clinical spectrum of disease. Hepatology. 1997;**26**(3 Suppl 1):15S-20S. DOI: 10.1002/hep.510260703

[3] Pockros PJ. Developments in the treatment of chronic hepatitis C. Expert Opinion on Investigational Drugs. 2002;**11**(4):515-528. DOI: 10.1517/13543784.11.4.515

[4] Strader DB, Wright T, Thomas DL, Seeff LB, American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C. Hepatology. 2004;**39**(4):1147-1171. DOI: 10.1002/hep.20119

[5] Dorr RT. Interferon-alpha in malignant and viral diseases: A review. Drugs. 1993;**45**(2): 177-211

[6] Miyaoka H, Otsubo T, Kamijima K, Ishii M, Onuki M, Mitamura K. Depression from interferon therapy in patients with hepatitis C. The American Journal of Psychiatry. 1999;**156**(7): 1120. DOI: 10.1176/ajp.156.7.1120

[7] Musselman DL, Lawson DH, Gumnick JF, Manatunga AK, Penna S, Goodkin RS, et al. Paroxetine for the prevention of depression induced by high-doseinterferon alfa. The New England Journal of Medicine. 2001;**344**(13):961-966. DOI: 10.1056/NEJM200103293441303

[8] Bonaccorso S, Marino V, Puzella A, Pasquini M, Biondi M, Artini M, et al. Increased depressive ratings in patients with hepatitis C receiving interferon-alpha-based immunotherapy are related to interferon-alpha-induced changes in the serotonergic system. Journal of Clinical Psychopharmacology. 2002;**22**(1):86-90

[9] Fischer CW, Eskelund A, Budac DP, Tillmann S, Liebenberg N, Elfving B, et al. Interferon-alpha treatment induces depression-like behaviour accompanied by elevated hippocampal quinolinic acid levels in rats. Behavioural Brain Research. 2015;**293**:166-172. DOI: 10.1016/j.bbr.2015.07.015

[10] Raison CL, Dantzer R, Kelley KW, Lawson MA, Woolwine BJ, Vogt G, et al. CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: relationship to CNS immune responses and depression. Molecular Psychiatry. 2010;**15**(4):393-403. DOI: 10.1038/mp.2009.116

[11] Steiner J, Walter M, Gos T, Guillemin GJ, Bernstein HG, Sarnyai Z, et al. Severe depression is associated with increased microglial quinolinic acid in subregions of the anterior cingulate gyrus: evidence for an immune-modulated glutamatergic neurotransmission? Journal of Neuroinflammation. 2011;**8**:94. DOI: 10.1186/1742-2094-8-94

[12] Chung RT, Gale M Jr, Polyak SJ, Lemon SM, Liang TJ, Hoofnagle JH. Mechanisms of action of interferon and ribavirin in chronic hepatitis C: Summary of a workshop. Hepatology. 2008;**47**(1):306-320. DOI: 10.1002/hep.22070

[13] Takaoka A, Yanai H. Interferon signalling network in innate defence. Cellular Microbiology. 2006;**8**(6):907-922. DOI: 10.1111/j.1462-5822.2006.00716.x

[14] Gale M Jr, Foy EM. Evasion of intracellular host defence by hepatitis C virus. Nature 2005;**436**(7053):939-945, DOI: 10.1038/nature04078.

[15] Sen GC. Viruses and interferons. Annual Review of Microbiology. 2001;**55**:255-281. DOI: 10.1146/annurev.micro.55.1.255

[16] Cook DN, Pisetsky DS, Schwartz DA. Toll-like receptors in the pathogenesis of human disease. Nature Immunology. 2004;**5**(10):975-979. DOI: 10.1038/ni1116

[17] Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. Nature Immunology. 2004;**5**(10):987-995. DOI: 10.1038/ni1112

[18] McCormick CJ, Challinor L, Macdonald A, Rowlands DJ, Harris M. Introduction of replication-competent hepatitis C virus transcripts using a tetracycline-regulable baculovirus delivery system. The Journal of General Virology. 2004;**85**(Pt 2):429-439. DOI: 10.1099/vir.0.19676-0

[19] Sumpter R Jr, Loo YM, Foy E, Li K, Yoneyama M, Fujita T, et al. Regulating intracellular antiviral defense and permissiveness to hepatitis C virus RNA replication through a cellular RNA helicase, RIG-I. Journal of Virology 2005;**79**(5):2689-2699, DOI: 10.1128/JVI.79.5.2689-2699.2005

[20] Li K, Chen Z, Kato N, Gale M Jr, Lemon SM. Distinct poly(I-C) and virus-activated signaling pathways leading to interferon-beta production in hepatocytes. The Journal of Biological Chemistry. 2005;**280**(17):16739-16747. DOI: 10.1074/jbc.M414139200

[21] Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, et al. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nature Immunology. 2004;**5**(7):730-737. DOI: 10.1038/ni1087

[22] Fitzgerald KA, McWhirter SM, Faia KL, Rowe DC, Latz E, Golenbock DT, et al. IKKepsilon and TBK1 are essential components of the IRF3 signaling pathway. Nature Immunology. 2003;**4**(5):491-496. DOI: 10.1038/ni921

[23] Kanazawa N, Kurosaki M, Sakamoto N, Enomoto N, Itsui Y, Yamashiro T, et al. Regulation of hepatitis C virus replication by interferon regulatory factor 1. Journal of Virology. 2004;**78**(18):9713-9720. DOI: 10.1128/JVI.78.18.9713-9720.2004

[24] Murray PJ. The JAK-STAT signaling pathway: input and output integration. Journal of Immunology. 2007;**178**(5):2623-2629

[25] Honda K, Takaoka A, Taniguchi T. Type I interferon [corrected] gene induction by the interferon regulatory factor family of transcription factors. Immunity. 2006;**25**(3):349-360. DOI: 10.1016/j.immuni.2006.08.009

[26] Menkes DB, MacDonald JA. Interferons, serotonin and neurotoxicity. Psychological Medicine. 2000;**30**(2):259-268

[27] Capuron L, Ravaud A, Neveu PJ, Miller AH, Maes M, Dantzer R. Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. Molecular Psychiatry. 2002;**7**(5):468-473. DOI: 10.1038/sj.mp.4000995

[28] Hassanain HH, Chon SY, Gupta SL. Differential regulation of human indoleamine 2,3-dioxygenase gene expression by interferons-gamma and -alpha. Analysis of the regulatory region of the gene and identification of an interferon-gamma-inducible DNA-binding factor. The Journal of Biological Chemistry. 1993;**268**(7):5077-5084

[29] Lawson MA, Parrott JM, McCusker RH, Dantzer R, Kelley KW, O'Connor JC. Intracerebroventricular administration of lipopolysaccharide induces indoleamine-2,3-dioxygenase-dependent depression-like behaviors. Journal of Neuroinflammation. 2013;**10**:87. DOI: 10.1186/1742-2094-10-87

[30] O'Connor JC, Andre C, Wang Y, Lawson MA, Szegedi SS, Lestage J, et al. Interferon-gamma and tumor necrosis factor-alpha mediate the upregulation of indoleamine 2,3-dioxygenase and the induction of depressive-like behavior in mice in response to

bacillus Calmette-Guerin. The Journal of Neuroscience. 2009;**29**(13):4200-4209. DOI: 10.1523/JNEUROSCI.5032-08.2009

[31] O'Connor JC, Lawson MA, Andre C, Briley EM, Szegedi SS, Lestage J, et al. Induction of IDO by bacille Calmette-Guerin is responsible for development of murine depressive-like behavior. Journal of Immunology. 2009;**182**(5):3202-3212. DOI: 10.4049/jimmunol.0802722

[32] O'Connor JC, Lawson MA, Andre C, Moreau M, Lestage J, Castanon N, et al. Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. Molecular Psychiatry. 2009;**14**(5):511-522. DOI: 10.1038/sj.mp.4002148

[33] Salazar A, Gonzalez-Rivera BL, Redus L, Parrott JM, O'Connor JC. Indoleamine 2,3-dioxygenase mediates anhedonia and anxiety-like behaviors caused by peripheral lipopolysaccharide immune challenge. Hormones and Behavior. 2012;**62**(3):202-209. DOI: 10.1016/j.yhbeh.2012.03.010

[34] Vecsei L, Beal MF. Influence of kynurenine treatment on open-field activity, elevated plus-maze, avoidance behaviors and seizures in rats. Pharmacology, Biochemistry, and Behavior. 1990;**37**(1):71-76

[35] Wichers MC, Koek GH, Robaeys G, Verkerk R, Scharpe S, Maes M. IDO and interferon-alpha-induced depressive symptoms: a shift in hypothesis from tryptophan depletion to neurotoxicity. Molecular Psychiatry. 2005;**10**(6):538-544. DOI: 10.1038/sj.mp.4001600

[36] Malaguarnera M, Laurino A, Di Fazio I, Pistone G, Castorina M, Guccione N, et al. Neuropsychiatric effects and type of IFN-alpha in chronic hepatitis C. Journal of Interferon & Cytokine Research. 2001;**21**(5):273-278. DOI: 10.1089/107999001300177457

[37] Ogawa S, Fujii T, Koga N, Hori H, Teraishi T, Hattori K, et al. Plasma L-tryptophan concentration in major depressive disorder: new data and meta-analysis. The Journal of Clinical Psychiatry. 2014;**75**(9):e906-e915. DOI: 10.4088/JCP.13r08908

[38] Teraishi T, Hori H, Sasayama D, Matsuo J, Ogawa S, Ishida I, et al. Personality in remitted major depressive disorder with single and recurrent episodes assessed with the Temperament and Character Inventory. Psychiatry and Clinical Neurosciences. 2015;**69**(1):3-11. DOI: 10.1111/pcn.12218

[39] Johansson AS, Owe-Larsson B, Asp L, Kocki T, Adler M, Hetta J, et al. Activation of kynurenine pathway in ex vivo fibroblasts from patients with bipolar disorder or schizophrenia: cytokine challenge increases production of 3-hydroxykynurenine. Journal of Psychiatric Research. 2013;**47**(11):1815-1823. DOI: 10.1016/j.jpsychires.2013.08.008

[40] Savitz J, Drevets WC, Smith CM, Victor TA, Wurfel BE, Bellgowan PS, et al. Putative neuroprotective and neurotoxic kynurenine pathway metabolites are associated with hippocampal and amygdalar volumes in subjects with major depressive disorder. Neuropsychopharmacology. 2015;**40**(2):463-471. DOI: 10.1038/npp.2014.194

[41] Carlin JM, Borden EC, Sondel PM, Byrne GI. Biologic-response-modifier-induced indoleamine 2,3-dioxygenase activity in human peripheral blood mononuclear cell cultures. Journal of Immunology. 1987;**139**(7):2414-2418

[42] Werner-Felmayer G, Werner ER, Fuchs D, Hausen A, Reibnegger G, Wachter H. Induction of indoleamine 2,3-dioxygenase in human cells in vitro. Advances in Experimental Medicine and Biology. 1991;**294**:505-509

[43] Mitsui M, Nishikawa M, Zang L, Ando M, Hattori K, Takahashi Y, et al. Effect of the content of unmethylated CpG dinucleotides in plasmid DNA on the sustainability of transgene expression. The Journal of Gene Medicine. 2009;**11**(5):435-443. DOI: 10.1002/jgm.1317

[44] Comai S, Cavalletto L, Chemello L, Bernardinello E, Ragazzi E, Costa CV, et al. Effects of PEG-interferon alpha plus ribavirin on tryptophan metabolism in patients with chronic hepatitis C. Pharmacological Research. 2011;**63**(1):85-92. DOI: 10.1016/j.phrs.2010.10.009

[45] Barton DA, Esler MD, Dawood T, Lambert EA, Haikerwal D, Brenchley C, et al. Elevated brain serotonin turnover in patients with depression: Effect of genotype and therapy. Archives of General Psychiatry. 2008;**65**(1):38-46. DOI: 10.1001/archgenpsychiatry.2007.11

[46] Sullivan GM, Oquendo MA, Huang YY, Mann JJ. Elevated cerebrospinal fluid 5-hydroxyindoleacetic acid levels in women with comorbid depression and panic disorder. The International Journal of Neuropsychopharmacology. 2006;**9**(5):547-556. DOI: 10.1017/S1461145705006231

[47] Murakami Y, Ishibashi T, Tomita E, Imamura Y, Tashiro T, Watcharanurak K, et al. Depressive symptoms as a side effect of Interferon-alpha therapy induced by induction of indoleamine 2,3-dioxygenase 1. Scientific Reports. 2016;**6**:29920. DOI: 10.1038/srep29920

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