Fish and Shellfish Immunology 93 (2019) 801–814

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Is related the hematopoietic stem cells differentiation in the Nile tilapia with GABA exposure?

Armando Vega-López[a,*], Nataraj S. Pagadala[b], Brenda P. López-Tapia[a], Ruth L. Madera-Sandoval[a], Erika Rosales-Cruz[c], Minerva Nájera-Martínez[a], Elba Reyes-Maldonado[c]

[a] *Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Laboratorio de Toxicología Ambiental, Av. Wilfrido Massieu S/n, Unidad Profesional Zacatenco, México, CP 07738, Mexico*

[b] *Cheminger Inc, 2131, 51stSW, Edmonton, Alberta, T6X 1A4, Canada*

[c] *Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Laboratorio de Citología, Carpio y Plan de Ayala S/n, Casco de Santo Tomás, México, CP 11340, Mexico*

ARTICLE INFO

Keywords:
c-kit
Sca-1
Molecular docking methodology
Neuroendocrinology
Oreochromis niloticus
Pluripotent stem cells
Hematopoietic stem cells

ABSTRACT

The signaling mediated by small non-proteinogenic molecules, which probably have the capacity to serve as a bridge amongst complex systems is one of the most exiting challenges for the study. In the current report, stem cells differentiation of the immune system in Nile tilapia treated with sub-basal doses of GABA evaluated as c-kit$^+$ and Sca-1$^+$ cells disappearance on pronephros, thymus, spleen and peripheral blood mononuclear cells by flow cytometry was assessed. Explanation of biological response was performed by molecular docking approach and multiparametric analysis. Stem cell differentiation depends on a delicate balance of negative and positive interactions of this neurotransmitter with receptors and transcription factors involved in this process. This in turn depends on the type of interaction with hematopoietic niche to differentiate into primordial, early or late hematopoiesis as well as from the dose delivery. In fish treated with the low doses of GABA (0.1% over basal value) primordial hematopoiesis is regulated by interaction of glutamate (Glu) with the Ly-6 antigen. Early hematopoiesis was influenced by the bond of GABA near or adjacent to turns of FLTR3-Ig-IV domain. During late hematopoiesis, negative regulation by structural modifications on PU.1/IRF-4 complex, IL-7Rα and GM-CSFR mainly prevails. Results of molecular docking were in agreement with the percentages of the main blood cells lineages estimated in pronephros by flow cytometry. Current study provides the first evidences about the role of inhibitory and excitatory neurotransmitters such as GABA and Glu, respectively with the most transcriptional factors and receptors involved on hematopoiesis in adult Nile tilapia.

1. Introduction

The ability of cells and organs to maintain homeostasis relies upon the accurate balance of regulatory mechanisms, particularly when the effectors organs depend on the signals made by soluble molecules. A suitable example is the central nervous system, which transduces information from the environment and organizes the integral response of the different organs to the body effectors [1]. The hypothalamo-hypophysial system is a member of the sympathetic nervous system regulated by feedback signals from the endocrine glands and other circulating factors [2–4]. Together with the adrenal glands, thyroid, gonads and liver encompass the named neuroendocrine system (NES) characterized by pulsatile release of a number of neurohormones, neuropeptides and hormones, midst others [5]. However, the integration of these signaling is a quite complex and the network between the neuroendocrine cells and amongst others cells permits a fine control to achieve homeostasis. This fact is possible to occur considering the entry of hormones and other factors into the brain through the cerebrospinal fluid [4]. Also, the ingress of cytokines, chemokines, prostaglandins (EP_3) and PAMP/DAMP to the brain and glands of the NES allow the bi-directional communication between nervous and immune systems [6–8]. This phenomenon is likely to take place because of presence of "hard-wired" circuitry of the autonomic nervous system with the immune system (IS) through nerve fibers located in primary and secondary lymphoid organs, and by existence of different types of neuroendocrine receptors on immunocompetent cells [8]. In addition, some

---

[*] Corresponding author.
E-mail addresses: avegadv@yahoo.com.mx, avegalo@ipn.mx (A. Vega-López).

https://doi.org/10.1016/j.fsi.2019.08.032
Received 14 March 2019; Received in revised form 7 August 2019; Accepted 12 August 2019
Available online 13 August 2019
1050-4648/

IS cells can produce neurotransmitters or possess the feedback mechanisms involved in the response mediated by such substances. Under certain stimuli, T cells and macrophages could synthesize γ-aminobutyric acid (GABA), and express receptor type A of the GABAergic transmission output machinery [9,10]. In other case, activation of somatosensory neurons, as example by exposure to PAMP/DAMP, induce action potentials that move from the brainstem to the spleen and other organs of the IS [11,12]. As a consequence of action potential differences, T cell release acetylcholine (ACh) able to interact with nicotinic acetylcholine receptor subunit alpha7 (α7 nAChR) present on immunocompetent cells to inhibit cytokine release carried out by macrophages [11,13]. If those complex networks between the NES and IS are amazing, others less recognized roles are documented. Neurotransmitters and neuropeptides could be potentially involved on hematopoiesis, priming and migration of immune cells [14]. Through pretty well performed experiments, it has been shown that the embryonic pluripotent cells express the GABAergic system and also spontaneously release GABA suggesting that those cells are able to establish a GABA niche by release of this transmitter [15]. Despite the outstanding findings previously documented, in juvenile and adult organisms a lack of information prevails about the possible role of GABA increase on differentiation of hematopoietic stem cells (HSCs). These cells are $\text{Lin}^{-}\text{c-kit}^{+}\text{Sca-1}^{+}$ [16] and inhabit the named LSK niche [17,18]. In the current study, the Nile tilapia (*Oreochromis niloticus*) was selected as a model because the IS of fish species contains both, the innate and adaptive response as in mammals [19]. However, the most important reasons were whether to known if develop of HSCs in bony fish respond to the stimuli elicited by neurotransmitters such as GABA and because representative *O. niloticus* genome assemblies exist (BioProject: PRJNA354796). Considering the former statements, in this study changes on surface markers (c-kit, and Sca-1) of the HSCs elicited by increase of GABA at sub-basal levels was evaluated by flow cytometry in cells of pronephros (the evolutionary equivalent to the bone marrow in mammals), spleen, thymus and peripheral blood mononuclear cells of the Nile tilapia (*Oreochromis niloticus*) after an eight days intravenous treatment. The interactions of this neurotransmitter with receptors and transcription factors involved on differentiation of HSCs were assessed by molecular docking methodology to explain the effects elicited by this substance and mathematical model for discriminate determinants interactions involved on HSCs differentiation.

2. Materials and methods

2.1. Specimens

Pre-adult specimens of Nile tilapia weighing 234 ± 18 g obtained from a fish farm were used in the present study. The specimens were maintained in the laboratory for three months before the beginning of the experiments using synthetic water (0.22 g $MgSO_4$, 0.18 g $NaHCO_3$, 0.08 g KCl and 0.13 g $CaSO_4{\cdot}2H_2O$ per L). In addition, photoperiods of 18 h light and 8 h dark, at 25 ± 2 °C, with aeration and constant filtration were maintained. The fish were fed with pellets for tilapia feed (Purina™).

2.2. Evaluation of γ–aminobutyric acid (GABA) basal levels

Prior to the exposure, basal levels of GABA were evaluated in the serum of ten tilapia by liquid chromatography with a Shimadzu HPLC equipment connected to a variable wavelength UV detector with a 5-µm C18 Agilent Technologies column (4.6 × 250 mm) using 2-hydroxynaphthaldehyde as derivatizing reagent [20]. In brief, 0.8 ml of serum was mixed with 0.8 ml of methanol HPLC grade (Sigma-Aldrich™), mixed and centrifuged at 12,000 rpm. Then, 0.7 ml of supernant, 0.6 ml borax solution (5 g/dL) pH 8.0 (Sigma ™) and 2.0 ml of derivatizing reagent (2-hydroxy-1-naphthaldehyde 0.3 w/v in methanol, Aldrich ™) was transferred to a glass tube with flat bottom, heated (80 °C/10 min) and cooled at room temperature. The mix was placed on 5 ml volumetric flask and the final volume was adjusted to 5 ml with methanol. Five µl of the mixture was injected in the C18 column using a solution of methanol:water (62:38 v/v) as mobile phase with a flow-rate 0.2 ml/min. Content of GABA in serum samples was estimated with a calibration curve of 4.68–75 µg/ml of γ-aminobutyric acid certified reference standard, TraceCERT® (Sigma-Aldrich™). Standout that it is possible to determine the absorbance at 330 nm of derivatized GABA in a microplate reader once the linearity of the method was verified by liquid chromatography following the steps detailed in brief.

2.3. Treatments

Considering basal levels of neurotransmitters, different doses of GABA dissolved in sterile injectable water were tested intravenously into the caudal vein to find the definitive test intervals. The acceptance criteria were the lack of mortality in 24 h post exposure or alterations in the behavior of the fish. Above 15% of sub-basal increases of GABA, 100% mortality was found in less than 24 h (data not shown). Two days before the treatment, the specimen was placed per treatment (n = 6) in a 140 L glass aquarium with dark walls and thick stone substrate to avoid behavioral alterations. Serial dilutions of this neurotransmitter were performed in a sterile injected water to reach the appropriate concentrations. GABA was dosed in three independent experiments at 0, 0.0048, 0.048 and 0.48 µg GABA/100 g fish, which represent the 0.1%, 1.0% and 10.0% of increase over the basal levels of GABA. The volume injected into the caudal vein of each solution was 50 µl at day zero, three and six. On the eighth day, specimens were sedated by freezing on ice for sampling the blood from the caudal vein with heparinized syringe and after, the fish were euthanized by rapid freezing (15 min/−80 °C) according to local protocol for the production, protection and welfare of experimental animals [21] and in agreement with Article 38 and Chapter V of the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32010L0063). Necropsy was done on ice bed to obtain the head of the kidney (pronephros), thymus and spleen. The treatment was performed in a three independent experiments. Peripheral blood mononuclear cells (PBMCs) was isolated by density gradient with Ficoll Paque Plus (Sigma™), while the tissues was rinsed with sterile PBS1X at 4 °C, and carefully sieved on a Falcon™ nylon cell strainers (Thermo Fisher Scientific™) using a syringe plunger and cool PBS1X. These cells were finally purified by density gradient with Ficoll Paque Plus to discharge debris and erythrocytes. Cells were rinsed with sterile PBS1X at 4 °C and centrifuged (3200 rpm/4 °C/15 min) three times to detect c-kit and Sca-1 by flow cytometry.

2.4. Flow cytometry detection of c-kit and Sca-1

Antibodies against immunogens of mammals to detect c-kit and Sca-1 were used due to cross-reactivity with fish species was found [22,23] and non-availability of specific antibodies for the fish markers in the market assessed in this study. The anti-c-kit antibodies possess variations in sensitivity, but did not stain different cells than those expressing c-kit [22,24,25]. For c-kit, monoclonal anti–c-kit conjugated to FITC (11-1171-82, eBioscience™) was selected due to similar Ab was used in other fish species [22,26]. Rabbit polyclonal antibody to Sca1/Ly6A/E (ab198852, Abcam) contains the UPAR/Ly6 domain and cross reactivity in mouse, rat and humans was reported. However, only a previous report about detection of lymphocyte antigen 6 complex, locus A/E (Sca-1) in fish species (*Cyprinus carpio*) using polyclonal antibodies with cross-reactivity exist [22]. Staining the cells of the Nile tilapia was performed in the dark at 37 °C/1 h using the primary antibodies diluted at 1:5000 in PSB1X/Tween. In the case of Sca-1, a goat anti-rabbit IgG

H&L-Alexa Fluor® 488 (ab150077, Abcam) was used as the secondary antibody (1:5000). Three serial washes using PBS1X and centrifugation after the staining with primary or secondary Ab were performed in the dark. Finally, cells were fixed with 100 µl of paraformaldehyde (4%) and re-suspended with 200 µl of PBS1X for flow cytometry analysis. Labeled cells were acquired at 10,000 events in a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA), and data was processed by Cell Quest Pro software (Becton Dickinson, San Jose, CA).

2.5. Evaluation of lymphoid and myeloerythroid lineages-derived cells in pronephros after the treatments with GABA by flow cytometry

Using the dot-plots obtained from the former flow cytometry analysis, separation of progenitors, erythrocytes, lymphocytes, and myelomonocytes in pronephros was resolved by combined light-scatter profiles (forward scatter FSC and side scatter SSC: cell size and cellular granularity, respectively) based on a previous report [27].

2.6. Molecular docking analysis

Despite the lack of evidence in fish species about the receptors and transcriptional factors involved in differentiation of hematopoietic stem cells (HSCs) compared to adulthood of mammals, the *O. niloticus* genome assemblies (*Oreochromis niloticus* (assembly ASM185804v2) allowed modelling of protein structures *in silico*. In order to build the 3D models, a template (protein whose crystal structure has been elucidated) needed to be identified. Basic Local Alignment Search Tool (BLAST) search was performed to select the crystal structure of the closest homolog available in the Protein Data Bank (PDB) (www.pdb.org). The protein that showed the highest percent identity was selected as a template for construction of 3D models. The 3D models were generated using the homology modelling program Modeller 9v11 [28]. The Ramachandran's map using PROCHECK (Programs to Check the Stereo Chemical Quality of Protein Structures) [29] and ERRAT graphs [30] were used to analyze the built homology models. The WHAT IF program was used to analyze and also identify residue packing and atomic contact for the detection of bad packing of side chain atoms or unusual residue contacts [31]. The Z-score of Ramachandran's plot was predicted using the WHAT_CHECK software [32]. The quality and accuracy of 3D model was then validated using Verify3D [33]. The protein models generated were visualized using MOE software suite. Further, the 3D models were used for the identification of active sites and for the docking of ligands. The active site was predicted using an alpha shape algorithm to determine potential active sites in 3D protein structures using MOE site finder [34]. Docking studies of GABA and precursor (glutamic acid) [35] against the built homology models were performed using dock module implemented in MOE20 [34].

2.7. Statistical analysis

The distribution of the data was analyzed by Shapiro-Wilk normality test. Since the p values obtained for c-kit and Sca-1 expression were higher than > 0.1, a Gaussian distribution was assumed. Data of treatments with regard to control fish was analyzed by one-way ANOVA followed by a Dunnett and post hoc test, and by a Tukey's multiple comparison test to detect differences among treatments using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA). Results were considered significant at $p \leq 0.05$.). With the aim to discriminate real interactions of GABA on stem cells differentiation, a redundancy analysis (RDA) was performed in basal levels as well as in specimens treated with the low doses of this neurotransmitter, along with its precursor, glutamic acid (Glu) using the XLSTAT software for Excel.

3. Results

3.1. Levels of γ–aminobutyric acid (GABA) in control fish

Basal value of GABA in the Nile tilapia was found to be 14.96 ± 0.52 µg GABA/ml serum (n = 10) which represent a value of 4.98 ± 0.17 µg GABA/100 g fish considering a value of 3% of total blood (W/W) in the Nile tilapia (http://digeset.ucol.mx/tesis_posgrado/Pdf/Alfredo_Mena_Herrera.pdf).

3.2. Basal values of c-kit^{+} and Sca-1^{+} cells in hematopoietic organs and peripheral blood mononuclear cells

Basal values of c-kit^{+hi} cells (including lower–and–upper 95% of CI with of mean) were lower in pronephros (0.475% ± 0.159%; 0.415–0.534%) than in the spleen (0.939% ± 0.159%, $p < 0.001$; 0.798–1.08%), but was higher compared to PBMCs (0.337% ± 0.049%, $p < 0.01$; 0.319–0.356%) and thymus (0.276% ± 0.034%, $p < 0.001$; 0.249–0.302%). Sca-1^{+hi} cells were statistically ($p < 0.01$) higher in the spleen (0.538% ± 0.221%; 0.456–0.621%) than in pronephros (0.354% ± 0.037%, 0.340–0.368%); however, in the pronephros, the mean basal value of Sca-1^{+} cells were higher compared to PBMCs (0.261% ± 0.055%, $p < 0.001$; 0.208–0.276%) and thymus (0.197% ± 0.029%, $p < 0.001$, 0.169–0.209%).

3.3. c-kit^{+} and Sca-1 expression by exposure to γ-aminobutyric acid (GABA)

The expression of c-kit^{+} cells was diminished in pronephros (Fig. 1A [36]) and spleen (Fig. 1C) with regard to control group. In contrast, the higher dose of GABA (0.48 µg/100 g fish) elicited an increase of c-kit^{+} cells in thymus (Fig. 1B) and PBMCs (Fig. 1D). However, the low doses of GABA (0.0048 µg/100 g fish) also triggered a decrease of c-kit^{+} cells as noted in pronephros and spleen. The Sca-1^{+} cells obtained from the thymus were the most responsive to the effects elicited by sub-basal increase of GABA. Sca-1^{+} cells increased in specimens treated with higher dose of GABA, mostly in pronephros (Fig. 1A), thymus (Fig. 1B) in the spleen (Fig. 1C) and PBMCs (Fig. 1D). Nevertheless, the decrease of c-kit^{+} and Sca-1^{+} cells elicited by the low doses (0.0048 µg GABA/100 g fish) of this neurotransmitter is particularly relevant for the current study. Representative cytometry results are shown in Suppl. Fig. 1.

3.4. Major blood lineages in pronephros after the treatments with GABA

In the pronephros of control fish, the great percentage of blood cells was erythrocytes (59.16 ± 15.99%) followed by precursors (20.40 ± 12.09%), lymphocytes (11.26 ± 3.99%) and myelomonocytes (9.42 ± 2.42%) (Fig. 2A). However, by exposure to GABA *i.p.* dosed these percentages clearly changed in pronephros, particularly at the low and medium doses. In the low doses of GABA (0.0048 µg GABA/100 g fish), the percentage of precursors statistically decreased (0.25 ± 0.06%, $p \leq 0.001$), similarly than observed with lymphocytes (4.75 ± 1.32%, $p \leq 0.001$) and myelomonocytes (4.30 ± 2.06%, $p \leq 0.001$). Contrastingly, the erythrocytes notably increased (92.80 ± 2.39%, $p \leq 0.001$) (Fig. 2B). At 0.048 µg GABA/100 g fish, the percentage of precursors decreased by approximately a half (8.94 ± 5.06%, $p \leq 0.01$) compared to control fish; however, the most notable decrease was observed in the percentage of erythrocytes (5.44 ± 3.42%, $p \leq 0.001$) while lymphocytes (68.22 ± 14.28%, $p \leq 0.001$) and myelomonocytes (17.34 ± 10.70%, $p \leq 0.05$) increased regarding to controls (Fig. 2C). However, in the high doses of GABA (0.48 µg GABA/100 g fish) only a decrease about of 0.41-fold lower in the percentage of myelomonocytes (3.94 ± 1.11%, $p \leq 0.001$) was found (Fig. 2D). Representative dot plots (FSC/SSC) are

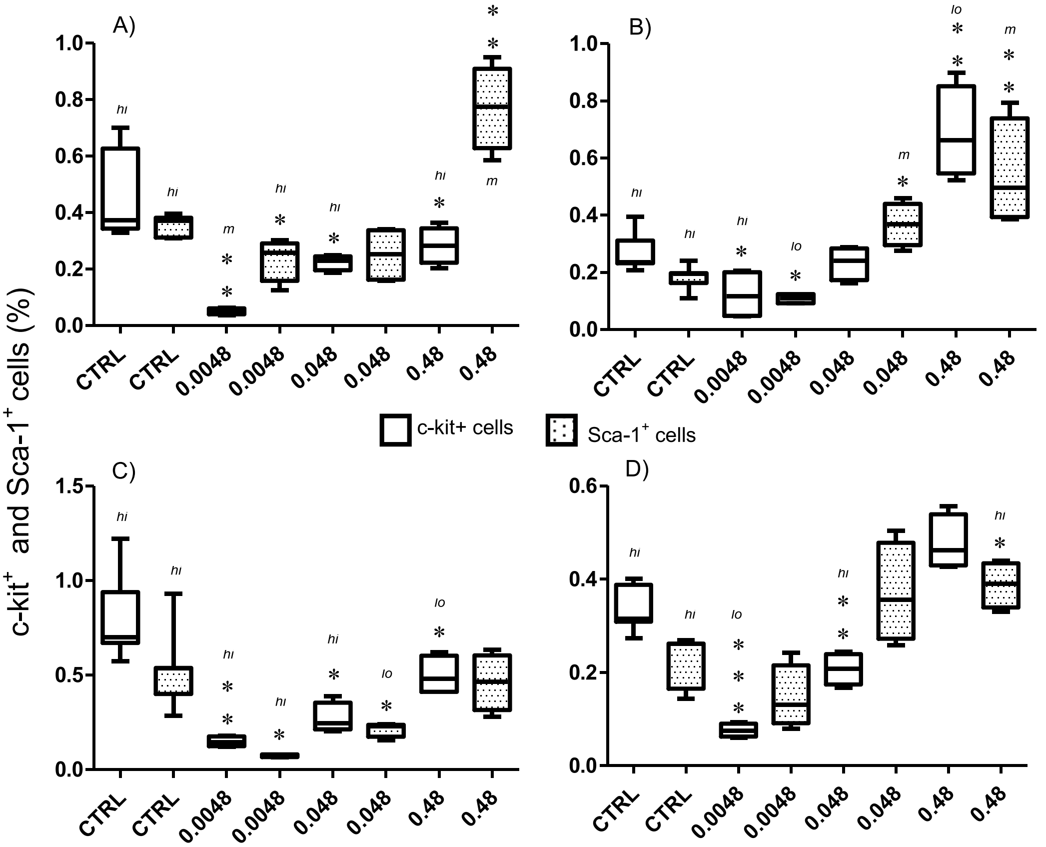

Fig. 1. Effects of sub-basal increase of γ-aminobutyric acid (GABA) on c-kit+ and Sca- 1 + immune system cells of the Nile tilapia (*Oreochromis niloticus*) disappearance evaluated by flow cytometry. Treatments with GABA (μg GABA/100 g fish): A) Pronephros. B) Thymus. C) Spleen. D) Peripheral blood mononuclear cells. Statistical differences regarding control fish with $^*p < 0.05$, $^{**}p < 0.01$. Mean fluorescent intensity (MFI) in treated fish: *hi* = high (≥5); *m* = mean (1–5); *lo* = low (< 1.0) obtained as MFI treated/MFI control [35]. In control fish MFI ≥800 is consider high.

show in Fig. 2E–H.

3.5. In silico analysis about effects of GABA and precursor (glutamic acid) on receptors and transcription factors involved on differentiation of pluripotent (PSCs) and hematopoietic stem cells (HSCs) of the Nile tilapia

In this study, receptors and transcription factors involved in differentiation of pluripotent and hematopoietic stem cells of the Nile tilapia are shown in Table 1 [37–65]. Docking studies of GABA (PubChem CID:119) and precursor (glutamic acid, PubChem CID:611) against the built homology models were performed using dock module implemented in MOE20. Using this strategy, it was found that these substances interact with all of these biomolecules (Suppl. Fig. 2). The results are challenge because a robust analysis was needed with the aim to discriminate real interactions of these neurotransmitters on stem cells differentiation evaluated as c-kit$^+$ and Sca-1$^+$ cells disappearance. Due to this, redundancy analysis (RDA) was performed in basal levels as well as in specimens treated with the low doses of GABA (0.0048 μg GABA/100 g fish) along with its precursor, the glutamic acid (Glu). Relations in the first and fourth quadrants represent key interactions able to maintain the immune system stem cells arrest, whereas in second and third quadrants show favorable relationships among neurotransmitters in the differentiation of these cells.

3.5.1. Effect of sub-basal doses of GABA and glutamic acid as in basal conditions on differentiation of pluripotent and hematopoietic stem cells

This study documented for the first time that increase in the sub-basal level of GABA could regulate hematopoietic stem cell differentiation through multiple interactions with apolar and polar residues, with strong H-bonding and long-range electrostatic interactions. Further observation by flow cytometry shows that PSCs and HSCs differentiation was favored by the low sub-basal increase of GABA (0.1% above of mean basal levels), which in turn explained with high variance (98.52%) in RDA (Fig. 3A). A value, which was higher than in control fish (87.44% of explained variance). Of this results, one section (Q1, Fig. 3A) shown that the arresting state (AS) of the pluripotent and hematopoietic stem cells, particularly c-kit$^+$ and Sca-1$^+$ cells of the spleen and PBMCs was maintained without GABA and Glu interactions. In contrast, diminished or absent GABA and Glu interactions with receptors and transcriptional factors of primordial, early and late hematopoiesis preserve the AS of c-kit$^+$ and Sca-1$^+$ cells in pronephros and thymus (Q4, Fig. 3A). During the primordial hematopoiesis null interactions of GABA with Ser32 (as side chain donor "SCD") and with Lys63 (as backbone donor "BBD") located on LU domain of the Ly-6 antigen (uPA receptor -like domain) allows the AS of these cells (Fig. 4A). Similar response was found when the contact of Glu217 (SCD) occurs on α5-helix of the canonical bundle of main fold of CXCR4 (Sca-1 receptor) of the Nile tilapia. In the same way, the glutamate also conserve the AS all through the primordial hematopoiesis as was the case of its contact with Ser133 (BBD) located on LMO2-like protein of LIM domain-containing proteins that bind with SCL:E47$_{\mathrm{hHLH}}$ heterodimer of T-cell acute lymphocytic leukemia protein (c-kit) (Fig. 4B). Also Glu in contact with Gly31 (BBD) of uPA receptor-like domain of Ly-6 antigen (Fig. 4C) and with Gly222 located of alpha-helical transmembrane protein of CXCR4 (Sca-1R) prevent the primordial hematopoiesis in pronephros and thymus. Interestingly, GABA and Glu interacts with some residues of receptors involved on early hematopoiesis, preserving in this way the AS. Minimum or absent contacts of GABA with Asn633 (BBD) present

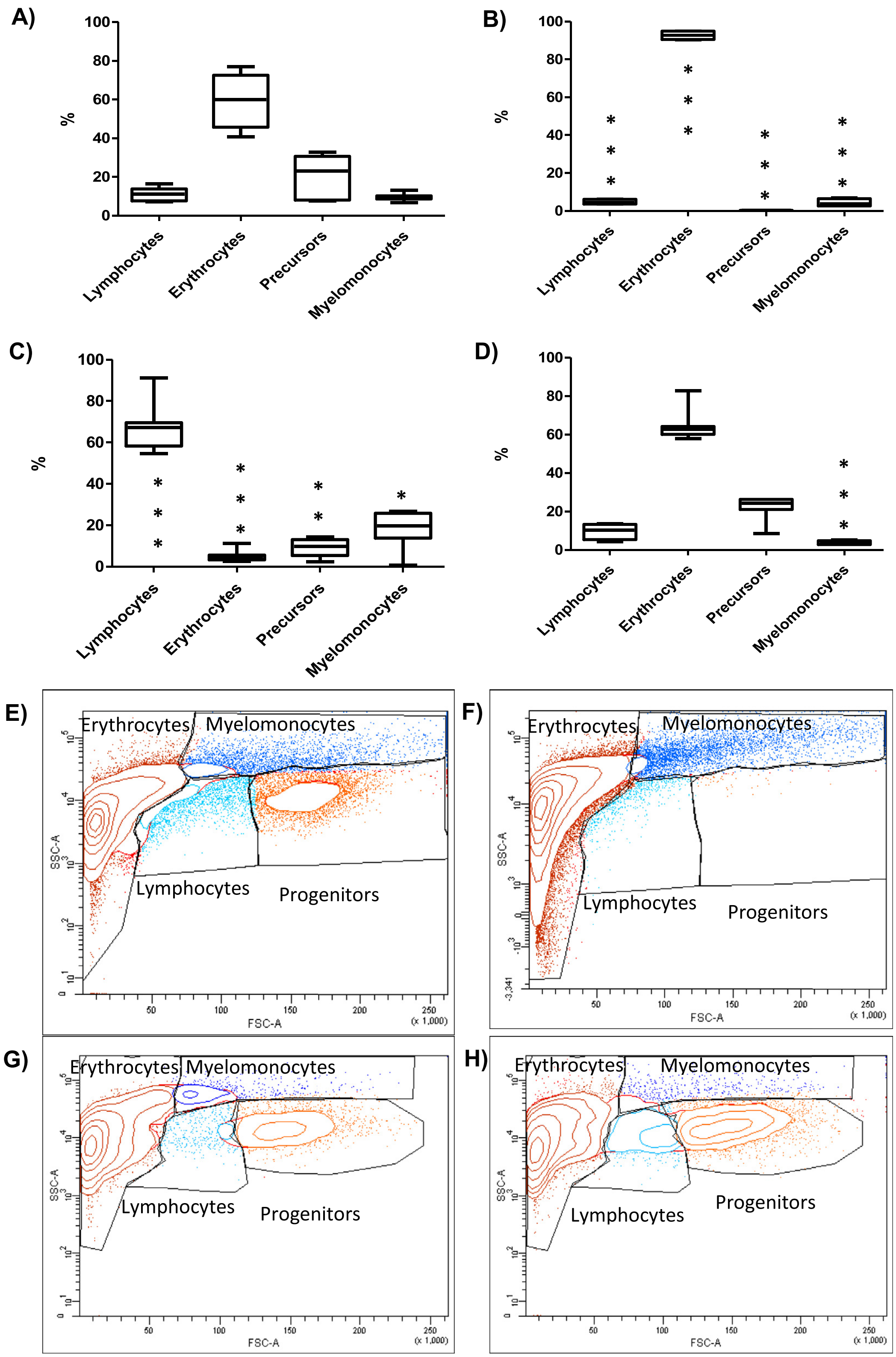

Fig. 2. Separation of major blood cell lineages (progenitors, erythrocytes, lymphocytes, and myelomonocytes) by combined light-scatter profiles (forward scatter FSC and side scatter SSC: cell size and cellular granularity, respectively) using flow cytometry in the pronephros of the Nile tilapia (*Oreochromis niloticus*) treated with sub-basal doses of GABA. **A)** Control fish. **B)** 0.0048 μg GABA/100 g fish. **C)** 0.048 μg GABA/100 g fish. **D)** 0.48 μg GABA/100 g fish. Representative dot plots in control fish **(E)** and in fish treated with 0.0048 μg GABA/100 g fish **(F)**, with 0.048 μg GABA/100 g fish **(G)** and with 0.48 μg GABA/100 g fish **(H)**. Statistical differences regarding control fish with $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

Table 1
Receptors and transcription factors probably involved on differentiation of hematopoietic stem cells (HSCs) and pluripotent stem cells (PSCs) of the Nile tilapia (*Oreochromis niloticus*).

NCBI Reference Sequence	Definition	Type of maker	Related function on mammals	Reference about function
Primordial hematopoiesis				
XP_003447931.2	T-cell acute lymphocytic leukemia protein 1 homolog (SCF or SCL)	c-kit (soluble and membrane-bound)	Growth factor/PSCs maintenance	[37[a], 38, 39]
XP_019206432.1	Prostate stem cell antigen	Sca-1 (Ly-6A.2/Ly-6E.1) (soluble and membrane-bound)	Growth factor/PSCs maintenance	[40[a]]
XP_005475984.1	C-X-C chemokine receptor type 4 (CXCR4)	Sca-1 receptor	PSCs maintenance/PSCs retention in the bone marrow/induce release CXCL12	[41, 42, 44[a]]
Early hematopoiesis				
XP_019218666.1	Receptor-type tyrosine-protein kinase FLT3	fms-like tyrosine kinase-3 receptor	ST-HSCs maintenance, proliferation and differentiation/HSCs maturation	[17, 42[a], 43, 44]
XP_019211737.1	Vascular cell adhesion protein 1 isoform X1	VCAM-1	Adhesion of LT-HSCs, ST-HSCs, MPP, LMPP and CMP/homing	[17, 38, 45[a], 46]
XP_005457175.1	Thrombopoietin receptor isoform X1	TPOR or C-Mpl Receptor	HSCs maintenance/HSCs self-renewed control/megakaryocyte and platelet production	[17, 39, 47[a]]
XP_003443202.1	Erythropoietin receptor	EPOR	Regulate cell-signaling of integrins and N-cadherins/activate bone marrow stromal cells/indirectly contribute to HSCs differentiation by signaling pathways	[48, 49, 50[a]]
Late hematopoiesis				
XP_005450299.1	GATA-binding factor 2 isoform X1	Binding factor	With ETV2 activate pan-myeloid or erythro-megakaryocytic programs/T lymphopoiesis/with SCF induce erythro-megakaryocytic differentiation	[17, 44, 51[a]]
XP_005450897.1	Transcription factor PU.1 isoform X1	PU.1 Factor	Lymphoid-primed MPP generation/formation of common lymphoid progenitors	[17, 52[a], 53, 54]
XP_003437930.1	Interferon regulatory factor 4 isoform X1	PU.1 Factor Receptor	Binder for the transcription factor PU.1/negative regulation of TLR signaling	[52[a], 53]
XP_003446873.1	PRED: thy-1 membrane glycoprotein	Differentiation antigen	Differentiation of MPP to T lymphopoiesis	[17, 55[a], 56, 57]
XP_005470653.1	Interleukin-7 receptor subunit alpha	IL-7 Receptor	LMP differentiation/HSCs self-renewal/regulate function of restricted progenitors/B lymphopoiesis	[39, 58, 59[a]]
XP_005454915.2	Polymeric immunoglobulin receptor isoform X1	Ig Receptor	LMP differentiation/B lymphoiesis/homing	[17, 60, 61, 62[a]]
XP_013133007.1	Macrophage colony-stimulating factor 1 receptor isoform X1	GM-CSF Receptor	HSCs maintenance and mobilization/increase myelopoiesis/lineage-instructive signal in primary bone marrow-derived GMP	[39, 63, 64[a], 65]

PRED: predicted. LT-HSCs: long term hematopoietic stem cells. ST-HSCs: short term hematopoietic stem cells. MPP: multipotent progenitors. LMP: lymphoid multipotent progenitor. CMP: common myeloid progenitor. GMP: granulocyte macrophage progenitors.

[a] References used for bioinformatics analysis and for deducing specifics domains in the Nile tilapia.

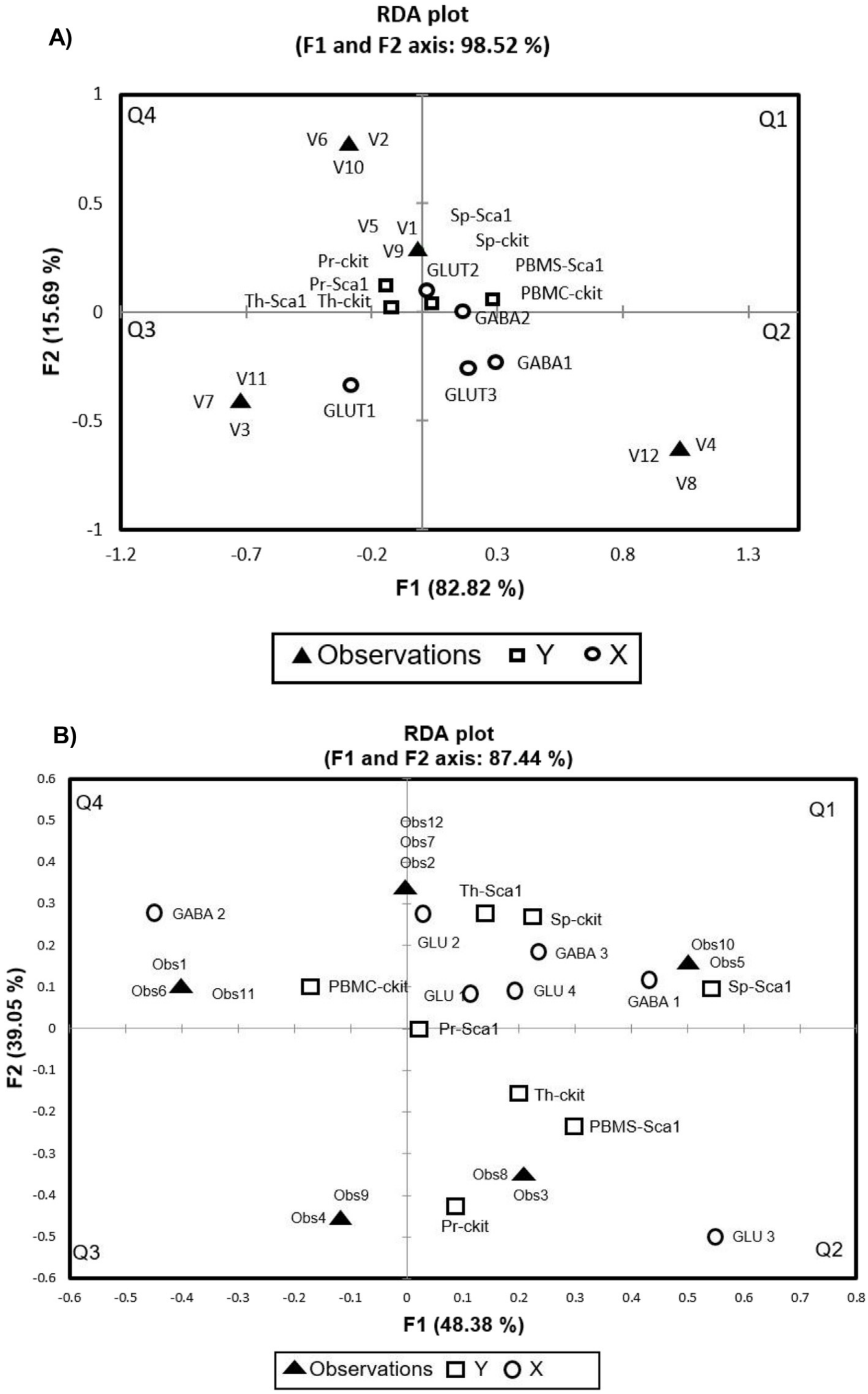

Fig. 3. RDA plots that represent binding energies (-kcal/mol) of γ-aminobutyric acid (GABA) and glutamate (Glu) as independent variables with receptors and transcriptional factors as dependent variables involved in differentiation of pluripotent and hematopoietic stem cells of Nile tilapia treated with sub-basal levels of GABA. Each quadrant (Qn) further represents the corresponding relationships. Capital letter G preceded by a diagonal indicates the specific relationships with Glu. **(A):** GABA-treated fish (0.0048 μg GABA/100 g fish) ***Q1***: No relations with GABA or Glu were found. ***Q2***: CXCR4-Gln217, EPOR-Trp60, VCAM1-Thr47, G/Lya6a-Pro27, G/GM-CSFR-Cys443, G/GM-CSFR-N-Met493. ***Q3***: G/ckit-Pro170, G/VCAMR-O-Glu49, G/FLT3-Thr377, G/EPOR-Gly68. ***Q4***: Lya6a-Ser32, Lya6a-Lys63, CXC4R-Glu217, TPOR-Asn633, EPOR-His126, PU-1-Glu133, PU.1-Leu168, PU.1R-Lys88, IgR-Ile410, IgR-Tyr411, GM-CSFR–OH–Thr494, G/c-kit-Ser133, G/Ly6a-Gly31, G/CXC4R -Gly222, G/FLT3-OH-His382, G/GM- EPOR-Glu65, G/EPOR-Gln72, G/EPOR-Arg120, G/GATA-Pro132, G/PU.1-Tyr130, G/PU.1-Pro171, G/PU.1R-Asp169, G/IL7R-Glu319, G/GM-CSFR-Trp517. **(B)**: Control fish, ***Q1***: Lya6a-Ser32, VCAM1-Phe15, IFR4-Ser133, IgR–OH–Ile410; G/FLT3-O-Ser353, G as anion/GM-CSFR-Trp494. ***Q2***: G/IFR-Arg167, G/IL7R-Glu319. ***Q3***: Lya6a-Gly31, FLT3R-Asp930, TPOR-Asn633, GATA-Glu201, IFR-Arg92, IL7R-Ser379; G/Lya6a-Gly31, G/FLT3R-His382, G/EPOR-O-Arg120, G/PU.1-Tyr130, G/IFR-Arg167, G/IL7R-N-Glu319, G as anion/GM-CSFR-Met493. ***Q4***: VCAM1-Thr47, EPOR–OH–Glu61, PU.1-Glu148. *Abbreviations:* Pr: pronephros. Th = thymus. Sp = spleen. PBMCs: peripheral blood mononuclear cells.

on CRH2 subdomain of IgR-like extracellular domain of TPOR (Fig. 4D) and with His126 (BBD) located between β6 and β7 of the D1 of N-terminal ectodomain (similar to the type FN3-like or Ig-2βR-like) of EPOR contributes also to maintain the AS of pronephros and thymus during the early hematopoiesis. Not only during the primordial and early hematopoiesis the bond of GABA play a role to preserve the AS of these cells, also do it during late hematopoiesis. Null or far away interactions of GABA with Glu133 (BBD) and Leu168 (BBD) present on PU.1-IRF-4 interface and with Lys88 (SCD) located on D1 of IL7Rα ectodomain allows the AS of c-kit$^+$ and Sca-1$^+$ cells of pronephros and thymus. Similar response was observed by contact of GABA with Ile410 (SCD) and with Tyr411 located in a loop between β30 and β31 of C-terminal domain of polymeric immunoglobulin receptor, and with Thr494 (SCD) present on the Ig domain of C-terminal domain of GM-CSFR.

Similarly, null or absent contacts of glutamate (Glu) contributes to maintain the AS during the early and late hematopoiesis in the pronephros and thymus of the Nile tilapia (Q4, Fig. 3A). During the early hematopoiesis, the hydroxylamine head of Glu acts as BBD with His382 present between β23 and β24 of FLT3R cytoplasmic domain (Fig. 4E), and with Glu65 (SCD), Gln72 (BBD), and Arg120 (SCD) located on the DI ectodomain (FN3-like or Ig-2βR-like) of EPOR diminishing this process in the pronephros and thymus. Likewise, the bond of Glu with some residues of transcriptional factors and receptors prevent the late

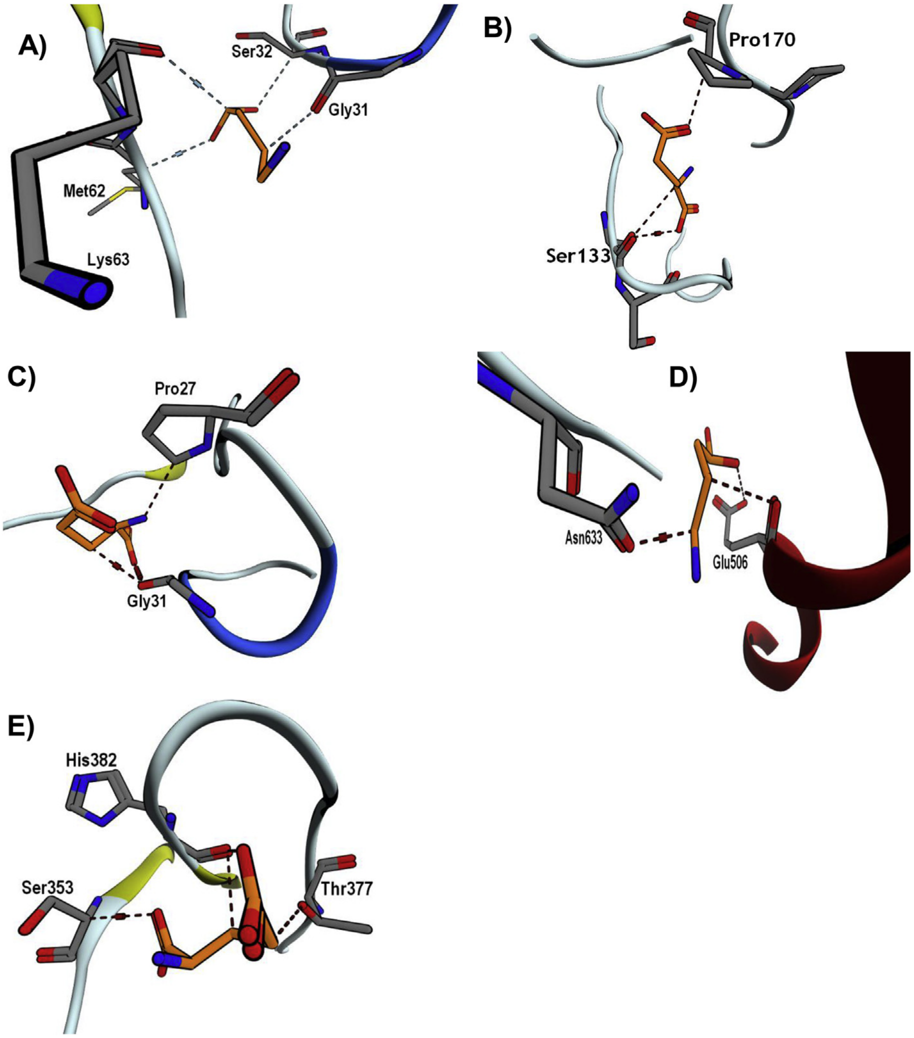

Fig. 4. Ligand-protein interactions that disallow the differentiation of pluripotent and hematopoietic immune system stem cells of Nile tilapia treated with sub-basal dose of GABA (0.0048 μg GABA/100 g fish). GABA with LU domain of the Ly-6 antigen (uPA receptor -like domain) **(A)**, glutamate with LMO2-like protein of LIM domain-containing proteins of T-cell acute lymphocytic leukemia protein (c-kit) **(B)**, glutamate with uPA receptor-like domain of Ly-6 antigen **(C)**, GABA with CRH2 subdomain of IgR-like extracellular domain of TPOR **(D)**, glutamate with FLT3R cytoplasmic domain **(E)** using MOE software suite (v2014).

hematopoiesis. Interaction of Glu with Pro132 (SCD) present on the complex N-domain of GATA binding factor 2, without having contact with DNA, with Tyr130 (SCD), Pro171 (SCD) located on IRF-4 conserved domain of PU.1/IRF-4/DNA complex, in addition to contact with Asp169 (SCD) present on D2 of IL7Rα ectodomain prevent the late hematopoiesis in the pronephors and thymus. In the same way, null interaction of glutamate with Glu319 (BBD) located in a loop between α3 and β19 cytoplasmic domain of IL7R and with Trp517 (H-*pi* interaction) present on Ig cytoplasmic domain of GM-CSFR favor the AS during late hematopoiesis in the pronephros and thymus of the Nile tilapia.

Contrasting with the negative regulation on primordial, early and late hematopoiesis that GABA and glutamate entails, there are significant bindings of GABA and Glu that favor the differentiation of PSCs and HSCs without an organ-specific response. These findings were corroborated by the measurement of c-kit$^+$ and Sca-1$^+$ cells disappearance detected by flow cytometry in fish treated with the low doses of GABA. Primordial hematopoiesis is favored by contact of GABA with Gln217 (BBD) located on the α5-helix of the binding pocket (CRS2) of helical domain of CXCR4 (Fig. 5A). Similarly, the bond of glutamate with Pro27 (BBD) present on the uPA receptor -like domain of Ly-6 antigen helps to sustain the same process (Fig. 5B). The early hematopoiesis increased by low doses of GABA, particularly if is in contact with Thr47 (SCD and BBD) located on the Ig-I domain (V-set) of VCAM-1 ectodomain (titin-like protein) (Fig. 5C), in addition to H-*Pi* interaction of Trp60 of D1 domain (N-ectodomain) of EPOR (minimum epitopic region) (Fig. 5D). However, the interactions of glutamate as glutamic acid elicit the late hematopoiesis by complex process that include electrostatics interactions of amidoxime head of glutamic acid as SCD with the Cys443 located on the single transmembrane domain and with Met93 of GM-CSFR (Fig. 5E) (Q2, Fig. 3A).

Opposing, it is evident in Q3 of Fig. 3A that scarce or null interactions of glutamate favors the primordial and early hematopoiesis in fish treated with the low doses of GABA. In the case of primordial hematopoiesis, null or absent bond of Glu with Pro170 (BBD) located in LMO2-like protein of c-kit allows this process. Similarly, the early hematopoiesis could be ideal if the bond of Glu linked with low doses of GABA is absent with Thr377 located between β23 and β24 of cytoplasmic domain of FLT3R, also when the hydroperoxyl head of Glu is in contact with Glu49 (BBD) present on the Ig-I domain ectodomain of VCAM or with Gly68 (SCD) of type FN3-like ectodomain of EPOR.

In control fish, predicted interactions show that of GABA and glutamic acid are able to regulate PSCs and HSCs differentiation with explained variance of 87.44% in RDA (Fig. 3B). Binding of GABA and Glu with transcription factors and receptors of primordial, early and late hematopoiesis are positive factors that contribute to maintain the AS of

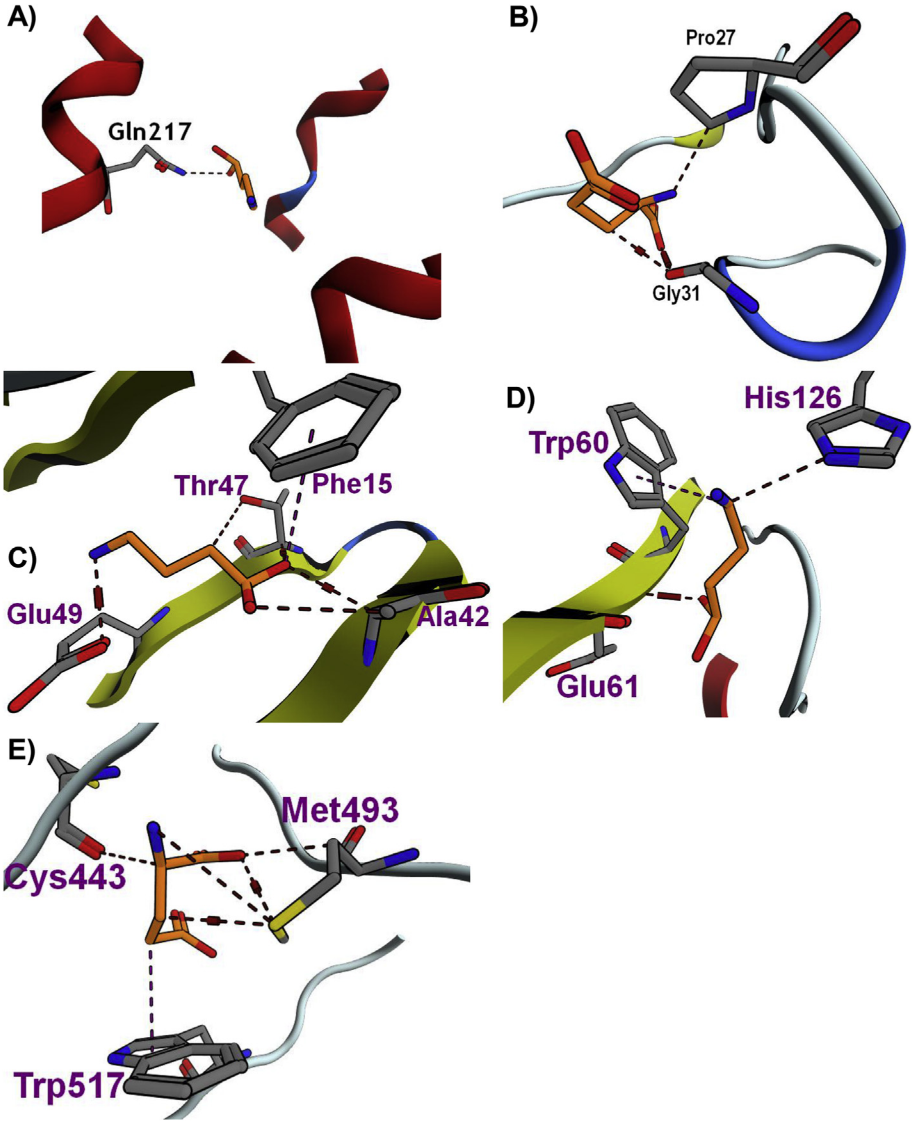

Fig. 5. Ligand-protein interactions that favor the differentiation of pluripotent and hematopoietic immune system stem cells of Nile tilapia treated with sub-basal dose of GABA (0.0048 μg GABA/100 g fish). GABA with binding pocket (CRS2) of helical domain of CXCR4 **(A)**, glutamate with uPA receptor -like domain of Ly-6 antigen **(B)**, GABA with Ig-I domain (V-set) of VCAM-1 ectodomain **(C)**, GABA with N-ectodomain of EPOR (minimum epitopic region) **(D)**, glutamic acid with single transmembrane domain of GM-CSFR **(E)** using MOE software suite (v2014).

Sca-1$^+$ cells of thymus and spleen as well as spleen and PBMCs c-kit$^+$ cells. Augmented interactions of GABA (BBD) with Ser32 of Ly-6 antigen, Phe15 (H-*Pi*) of VCAM-1 signal peptide, Ser133 (SCD) of IRF-4 conserved domain and Ile410 (BBD) of IgR cytoplasmic domain play a role in AS of spleen Sca-1$^+$ cells. Stronger interactions of Glu with Ser353 (SCD) of FLT3R Ig-IV domain and the glutamic acid (anionic form of glutamate) with Thr494 (BBD) of GM-CSFR on Ig-V domain allows the AS of thymus Sca-1$^+$ cells and spleen c-kit$^+$ cells (Q1 of Fig. 3B). GABA could elicit the AS of PBMCs c-kit$^+$ cells if is in contact with Thr47 (SCD and BBD) of VCAM-1 Ig-I domain, with Glu61 on D1 domain of FN3-like of EPOR and with Glu148 (BBD) of PU.1 factor (BBD) (Q4 of Fig. 3B).

In the opposite situation, diminished interactions of Glu with transcription factors of late hematopoiesis are promising scenario linked with differentiation of pronephros and thymus c-kit$^+$ cells as well as for PBMCs Sca-1$^+$ cells. If interactions of Glu (SCD) with Arg167 near to PU.1-IRF-4 interface and with Glu319 of IL-7R cytoplasmic domain are seen, the differentiation of these cells are allowed (Q2 of Fig. 3B), opposing to the found in pronephros and thymus of fish treated with the low doses of GABA just in the case of bonding of Glu with Glu319 of IL-7R. In contrast, the bonding of Glu as BBD with His382 present between β23 and β24 of FLT3R cytoplasmic domain allows the differentiation of pluripotent and hematopoietic stem cells without organ-specific response in control fish. The bonding of GABA (SCD) with Gly31 of Ly-6A.2/Ly-6E.1 antigen, Asn633 of TPOR, Asp930 of FLT3R cytoplasmic domain, Glu201 of GATA-binding factor 2 N-domain and with Ser379 of IL-7R cytoplasmic domain allows the differentiation of PSCs and HSCs surely occurs. Similar response might observe if GABA (BBD) is in contact with Arg92 of IRF-4 domain. In addition, Glu (SCD) might contribute to primordial and early hematopoiesis if its contacts with Gly31 of Ly-6A.2/Ly-6E.1 antigen and with His382 of FLT3R Ig-IV cytoplasmic domain. This response was concordant with the findings of fish treated with the low doses of GABA suggesting that the bonding of Glu with uPA receptor -like domain of Ly-6A.2/Ly-6E.1 antigen and with CRH2 subdomain of IgR-like extracellular domain of TPOR maintains the AS of primordial and early hematopoiesis. Likewise to the observed with the bonding of Glu with His382 of FLT3R Ig-IV cytoplasmic domain, which maintain the AS in pronephros and thymus of fish treated with the low doses of GABA. In control fish, bond of Glu as anion with Met493 of GM-CSFR Ig-V domain is not a favorable scenario for differentiation of c-kit$^+$ cells as well as for PBMCs Sca-1$^+$ cells in pronephros and thymus. Similar relationships of Glu as BBD were detected with Arg120 of D1 ectodomain of the EPOR, with Tyr130 of PU.1 factor (Q3 of Fig. 3B) as detected also in pronephros and thymus of fish treated with the low doses of GABA. In control fish, increased interaction of Glu with Arg167 of IFR and with Glu319 of IL7R could be a negative situation for the differentiation of c-kit$^+$ cells as well as for PBMCs Sca-1$^+$ cells in pronephros and thymus (Q3 of Fig. 3B) contrasting with the finding in fish treated with the low doses of GABA.

4. Discussion

For the first time in the current study, the complexity of the chemical-biological interactions of some neurotransmitters such as the case of GABA and glutamate capable of modulating the differentiation of pluripotent and hematopoietic stem cells was documented in Nile tilapia. Unlike other studies, it was shown that this process depends on a delicate balance of negative and positive interactions such as SCD or BBD with the receptors and transcription factors involved in primordial, early or late hematopoietic differentiation of PSCs and HSCs. Likewise, differentiation elicited by the low increase in the sub-basal levels depends not only on the neurotransmitter, but also of its precursor for GABA. Due to extended results, the interactions of these neurotransmitters in PSCs and HSCs differentiation were highlighted without mentioning of some studies performed in the Nile tilapia regarding erythropoiesis, granulopoiesis, lymphoplasmapoiesis and thrombopoiesis by ultrastructural examination [66]. Similarly, about the role of c-kit on PSC and HSC differentiation in *Danio rerio* and some amphibian's species [67,68].

There are consistent interactions of GABA and glutamate able to maintain the AS of PSCs and HSCs in control fish as well as in specimens treated with the low doses of GABA. Among receptors and transcriptional factors involved on PSCs and HSCs differentiation the role of T-cell acute lymphocytic leukemia protein 1 (c-kit or SCL) is widely recognized [37–39] and involves enhanced self-renewal, lineage, and reconstitution potentials during both steady state and stress hematopoiesis [69–72]. In fish treated with the low doses of GABA, the interactions of glutamate (excitatory neurotransmitter) with the LMO2-like protein of LIM domain-containing proteins of SCL contribute with the AS of Sca-1$^+$ cells and c-kit$^+$ cells in pronephros and thymus. Similarly, the null or absent interactions of Glu with LMO2-like protein could favor the differentiation of primordial hematopoietic cells without an organ-dependent response. These findings suggest that structural modifications on the LIM domain-containing proteins of SCL by contact with Glu is an obstacle for binding the SCL:E47$_{\mathrm{hHLH}}$ heterodimer with DNA-binding domain. Therefore, the activity of helix-loop-helix heterodimer DNA-binding domain to act as transcription factor is disrupted. Fascinatingly, bHLH E12/E47 that comprise LIM domain (LMO2:LDB1 complex), plays a role in rescuing functional hematopoiesis from SCL DNA-binding domain of adult non-lymphoid human cells [73]. In this way, it is feasible to reinforce the findings of the current study about the role of Glu by interactions with LMO2-like protein of SCL as negative forces for PSC and HSC differentiation in lymphoid organs of the Nile tilapia. Other well-documented transcriptional factor that regulates the primordial hematopoiesis by acting as growth factor and by its role on PSC maintenance is the Ly-6 antigen [40]. Interactions of GABA with LU domain of Ly-6 antigen (uPA receptor -like domain) are negative forces for c-kit$^+$ cells and Sca-1$^+$ cells differentiation in the thymus and spleen of control fish and in the pronephros and thymus of fish treated with the low doses of this neurotransmitter. Ly-6 antigen (Sca-1) is one of the characteristic markers of primordial stem cells and plays an important role in self-renewal and activation of progenitor stem cells [74,75]. Considering these findings, it is possible to suggest that interactions of the inhibitory neurotransmitter (GABA) on the GPI-linked cell-surface glycoprotein (as example the Ly-6 family) is an alternative for maintain the AS of PSCs on the main hematopoietic organs of the Nile tilapia. Despite preceding reports does not exists, also is relevant to consider the role of Glu on Ly-6 antigen. Interestingly, the exposure to the low doses of GABA favored the bond of Glu with uPA receptor—like domain of Ly-6 antigen allowing the primordial hematopoiesis without an organ-specific response. This response corroborate the relevance of balance between inhibitory/excitatory neurotransmitters, not only at cellular levels, but also at the level of hematopoietic niche as will be discussed later, enabling the differentiation of PSCs by interactions with Ly-6 antigen. Nevertheless, binding of Sca-1 with its receptor, the CXCR4 located on some cells of LSK niche is involved in the support of the survival, retention and/or differentiation of stem cells [38,39,76–82] via PI3K [83], Akt/Erk [84], STAT3/VEGF [85] and Wnt/Notch1 signaling [86]. In this regard, the contact of GABA with binding pocket of CXCR4 helical domain disallowed the primordial hematopoiesis in pronephros and thymus in fish treated with the low doses of GABA. The findings about the role of GABA and Glu on primordial hematopoiesis denote predominance of negative interactions at the level of the transcription factor “SCL” and on CXCR4. However, the excitatory neurotransmitter, the glutamate, may contribute with the differentiation of PSCs and HSCs by interactions with the Ly-6 antigen in the main immune system organs of the Nile tilapia. This finding was corroborated by statistical diminution of precursors analyzed by flow cytometry in the fish treated with the low doses of GABA.

The early hematopoiesis is regulated by receptors and adhesion proteins, mainly the receptor-type tyrosine-protein kinase of FLT3 (FLT3R named also “fms-like tyrosine kinase-3 receptor”) [42], the vascular cell adhesion protein 1 (VCAM-1) [45], the thrombopoietin receptor (TPOR or C-Mpl receptor) [47], and the erythropoietin receptor (EPOR) [50] among others. The low doses of GABA play a role mainly by negative regulation of the early hematopoiesis. The null or absent interactions of Glu with His382 as well as by bonding with Thr377 present between β23 and β24 of the cytoplasmic domain (Ig-IV domain) of FLT3R could favor the differentiation of c-kit$^+$ and Sca-1$^+$ cells in the pronephros and spleen. Similarly, in control fish this neurotransmitter functions as negative regulator in the differentiation of pluripotent and hematopoietic stem cells without organ-specific response. Regardless of a lack of information about the topic prevails; current results suggest that induced conformational change by interactions with Glu reduce the affinity of Ig-IV domain to bind with its substrate (FL). However, if the interaction of Glu occurs near or adjacent to turns, as example with Ser353, the structural organization of this domain occurs increasing the affinity with its substrate, improving consequently the differentiation of c-kit$^+$ cells in the spleen and Sca-1$^+$ cells is the thymus of control fish. Despite these findings, the bonding of GABA on FLT3R cytoplasmic domain (Asp930) disallowed the autophosphorylation and phosphorylation of downstream targets in control fish preventing in this way the differentiation of MMPs. Nevertheless, the high percentage of erythrocytes detected by flow cytometry in fish treated with the low doses of GABA suggest a gradual loss of FLTR3 expression required for differentiation of long-term HSCs (LT-HSCs) to reach MMP differentiation as documented in earlier studies for mammalian species [17]. The fish treated with the low doses of GABA the early hematopoiesis increased without an organ-dependent response by contact with Ig-I domain (V-set) of VCAM-1 ectodomain. This response may be an indication that adhesion of LT-HSCs, ST-HSCs, MPP, LMPP and CMP/homing take place [17,38,45,46]. Nevertheless, the hydroperoxyl head of excitatory neurotransmitter in contact with Glu49 of this Ig-I domain ectodomain elicit unfavorable conformational changes abolishing the differentiation of HSCs, MPPs, LMPPs and CMPs. However, in control fish, increased interaction of GABA on VCAM-1 signal peptide provoke conformational changes able to maintain the AS of spleen Sca-1$^+$ cells. Observed interactions of GABA and Glu on VCAM-1 are a clear example of the delicate balance that must prevails between the role of inhibitory and excitatory neurotransmitters involved on the early hematopoiesis. However, the increase of erythrocytes found by flow cytometry suggests the adhesion and posterior differentiation of multipotent progenitors. Contrasting with the previous findings, the bonding of GABA and Glu with thrombopoietin receptor put forward lower participation during the early hematopoiesis. In control fish, the bond of GABA and Glu with CRH2 subdomain of IgR-like extracellular domain of TPOR maintain the AS of pronephros and thymus during the early hematopoiesis. This interaction proposes that such modification reduce the affinity of TPOR with its natural substrate thrombopoietin. When this binding is successful helps in differentiation of stem cells by activation of JAK/STAT, Ras, and MAPK pathways in mammals [87–92]. Interestingly, the sequence motif "WSXWS" involved in receptor signaling close to transmembrane domain of Nile tilapia TPOR is likewise observed in humans as "WSDWS" [47]. Also in control fish, null or absent interaction of GABA at the end of B2 box motifs (Asn633) of C-terminal domain of TPOR maintains the AS of primordial and early hematopoiesis. This finding probably denotes that alteration of LEKL motif is able to disrupt the tyrosine phosphorylation mediated by this receptor. In this regard, the cytokine receptor motifs of TPOR such as PS, PPIP and LEKL involved in functional tyrosine phosphorylation are present in this fish species, standing out its role in differentiation of HSCs as found in mouse [91]. In specimens treated with the low doses of GABA, minimum or absent bond of GABA between β6 and β7 of the D1 of N-terminal ectodomain (similar to the type FN3-like or Ig-2βR-like) of EPOR contributes likewise to maintain the AS of pronephros and thymus during the early hematopoiesis. Similarly, null or absent interactions of Glu by contact with the same ectodomain of EPOR. In control fish, the interactions of GABA or Glu on this ectodomain are not a favorable scenario for differentiation of c-kit$^+$ cells. Despite preceding reports do not exists, these interactions propose that minimum epitopic region for productive ligand (EPO)-protein interactions present on D1 ectodomain of EPOR [50] is disrupted. The consequences of such bindings clearly indicate the existence of point control elicited neuroendocrine system involved on HSCs differentiation. Highlights that this receptor is involved on regulation of cell-signaling mediated by integrins and N-cadherins and in the activation of bone marrow stromal cells and indirectly contribute HSCs differentiation by different signaling pathways [48–50]. In contrast, in the fish treated with the low of GABA, only the H-*Pi* interaction of GABA with Trp60 present on the minimum epitopic region of D1 domain (N-ectodomain) of EPOR allows the differentiation of HSCs. Current finding is to some extent opposite to a previous study using small EPOR synthetic agonist (MW < 300 Da) which demonstrated that EPOR receptor asymmetry (not specific interactions such as salt bridges, H-bonds, and hydrophobic clusters) is the critical factor needed for activating signal transduction involved in human HSC differentiation via the JAK/STAT pathways [93]. However, by computational analysis was showed that such asymmetry is involved on JAK2 and MAPK/NF-κB pathways needed for the differentiation of erythropoietin-dependent erythroid progenitors [94,95]. Such findings are in agreement with the current study in which a high increase of erythrocytes was found by flow cytometry analysis in the Nile tilapia treated with the low doses of GABA.

In the late hematopoiesis, the role of factors, differentiation antigen and receptors are widely recognized such as the case of GATA-binding factor [27,51], transcription factor PU.1 [52], PU.1 factor receptor (interferon regulatory factor) [52], thy-1 membrane glycoprotein (differentiation antigen) [55], IL-7 receptor [59], Ig receptor [62] and GM-CSF receptor [64] among others. In the Nile tilapia, the role of GABA and Glu by interactions with GATA-binding factor is controversial. In fish treated with the low doses of GABA the interaction with Pro132 present on the complex N-domain of GATA binding factor 2 prevent the late hematopoiesis in the pronephros and thymus. Whereas, in control fish, the bond of this neurotransmitter with Glu201 present on the same domain allowed the differentiation of PSCs and HSCs of pronephros and thymus c-kit$^+$ cells as well as for PBMCs Sca-1$^+$ cells. This finding indicates that conformational change elicited by the low sub-basal increase of GABA on N-domain of GATA-binding factor is a negative factor for the proper binding of N-finger domain with GATA consensus site of DNA. However, more studies are required about the topic. Considering that the functionally transcription factor PU.1 is dependent of its binding with its receptor (IRF-4) [52], the role of GABA and Glu is a quite complex. In fish treated with the low doses of GABA, null or far away interactions of GABA with PU.1-IRF-4 maintain the AS of c-kit$^+$ and Sca-1$^+$ cells of pronephros and thymus. Similarly, interaction of Glu with the IRF-4 conserved domain of PU.1/IRF-4 prevents the late hematopoiesis in the pronephros and thymus as occurs also by bonding of GABA and Glu with IRF-4 present in the same organs. Likewise in control fish, an increased interaction of GABA with IRF-4 conserved domain is a key factor involved for maintaining the AS of spleen Sca-1$^+$ cells. These findings are an indication that structural modification on the PU.1/IRF-4 is an obstacle for lymphoid-primed MPP generation and for the formation of common lymphoid progenitors as reported under normal hematopoiesis in mammals [17,52–54]. The reduction of lymphocytes in fish treated with the low doses of GABA detected in the current study reinforce this hypothesis.

The specimens treated with the low doses of GABA show that diminished or absent GABA bond with IL-7Rα ectodomain as well as with C-terminal domain allows the AS of c-kit$^+$ and Sca-1$^+$ cells of pronephros and thymus. Similarly, null interaction of Glu with cytoplasmic domain of IL-7Rα favors the same response. In the case of control fish, the augmented interactions of GABA with the cytoplasmic domain of this receptor contribute on maintaining the AS of spleen Sca-1$^+$ cells, and Glu in contact with IL-7Rα elicit the same effect on differentiation of c-kit$^+$ cells as well as for PBMCs Sca-1$^+$ cells in pronephros and thymus. These interactions put forward that LMP differentiation and B lymphoiesis are deregulated by effect of the low doses of GABA, corroborating this finding by statistical diminution on the percentage of lymphocytes in the treated specimens. One of the processes involved on increase myelopoiesis and lineage-instructive signaling of granulocyte macrophage progenitors is regulated by the GM-CSFR [39,63–65]. In the Nile tilapia, the bond of GABA plays a role to preserve the AS of c-kit$^+$ cells and Sca-1$^+$ cells in pronephros and thymus by contact with Ig domain of C-terminal domain of GM-CSFR. Comparable response was noted in control fish in which the interactions of Glu with GM-CSFR Ig-V domain disallowed the differentiation of c-kit$^+$ cells as well as for PBMCs Sca-1$^+$ cells in pronephros and thymus. Besides, null H-*pi* interaction of Glu with Ig cytoplasmic domain of GM-CSFR favored the AS during late hematopoiesis in the pronephros and thymus of the Nile tilapia. These results put forward the structural modification elicited by GABA and Glu on GM-CSFR Ig-V domain which disallows the autophosphorylation of the two main sites of juxtamembrane cluster involved on the protein tyrosine activity of this receptor as documented during the normal hematopoiesis of mammals [64]. Contrasting, the electrostatics interaction of Glu, as glutamic acid, on the single transmembrane domain of GM-CSFR favors the late hematopoiesis in the Nile tilapia exposed to the low doses of GABA. The biological consequences of this finding remain to be proved; however, the significant reduction on the percentage of myelomonocytes found on this study suggest that disruption of autophosphorylation regulated by the protein tyrosine activity of GM-CSFR is the most probably effect elicited by sub-basal increase of GABA.

Notwithstanding that it is not possible to discern if the statistical interactions of GABA and Glu involved on PSCs and HSCs differentiation occurs in the hematopoietic niche or in the lymphoid organs such as the thymus or spleen, the flow cytometry analysis of the main blood cells lineages and the c-kit$^+$ and Sca-1$^+$ cells disappearance provides some clues. The molecular and cellular process involved in genesis, maturation and maintaining of PSCs and HSCs are widely conserved across vertebrates [96]. In the adult Nile tilapia, the kidney (pronephros and mesonephros) are the main sites for the lymphopoiesis/hematopoiesis; however, it is not possible to discharge the role of the spleen on this process [66]. Also, the peritoneum membrane is considered has a secondary function on this process by the presence of mesenchymal stem cells [66]. In the hematopoietic niche a lot of signaling pathways and extracellular matrix components is critical for HSC differentiation as well as the hematopoiesis is conserved across the species [96,97]. In the hematopoietic niche of mice, a lot of different cells types have a supportive role of HSCs such as endosteal cells, endothelial cells, perivascular cells, and mesenchymal stem cells (MSCs) [98]. In addition, during the embryonic development others supportive environments play a role such as aorta gonad mesonephros region which is responsible for HSC generation [97,99,100], whereas the fetal liver promotes HSC maturation and amplification [101,102]. However, in fish species embryo, remains to be proven which are the key cells present on the hematopoietic niche involved on HSC fate? [96].

By means of transcriptome analysis of stromal cell lines of mice Charbord team-work [98] demonstrated that the main genes positively correlated with stromal HSC-supportive capacity were *Ly6a*, *Ebf3, Col1a1*, and *Ncam1*, among others are present in best supportive stromal cell lines. Notwithstanding, in the current study we do not analyzed gene expression, the concordance of Ly6a antigen as the key factor involved on precursor disappearance is remarkable. Also the cell adhesion molecules (CAMs), characteristics of PSCs, are responsible for the localization of hematopoiesis to the bone marrow such as the case of neural cell adhesion molecule (*Ncam1*) and vascular cell adhesion protein VCAM due to its capacity to mediate the physical association between the developing hematopoietic cells and marrow stromal tissue [103]. In the current study, the bong of GABA with Ig-I domain of VCAM-1 ectodomain was positively correlated with increased early hematopoiesis, a finding which was in agreement with the transcriptome analysis of stromal cell lines of mice. Similarly, in the supportive HSC environments, highlighted the role of CAMs as the best correlated molecule involved on stromal HSC-supportive capacity [98]. In knockout zebrafish embryo, C-X-C chemokine receptor was correlated with functions as positive regulator of PSC and HSC colonization of the vascular niche [102]. Contrasting with these results, in the current study GABA and Glu play a negative regulation on primordial hematopoiesis at the level of CXCR4. The difference among both studies is an example how neurotransmitters modulate the PSC colonization in adult fish, which possess a developed vascular niche in addition to a mature neuroendocrine system, in contrast with fish embryo that support the early stages of hematopoietic development during the primitive wave of LSK cells [97]. Interestingly, it have been demonstrated the role of central nervous system on PSC and HSC development via the hypothalamic-pituitary-adrenal/interrenal stress response system. Increased *runx1* expression and *Flk1(+)/cMyb*(+) in PSCs and HSCs of zebrafish embryo was regulated by serotonin or by the reuptake of fluoxetine in an independent way of peripheral innervation [104]. With the basis of this report, and because in some cases GABA and Glu play a role on PSCs and HSCs differentiation without an organ-independent response, it is possible to propose that nervous innervation of hematopoietic niche in the adult Nile tilapia is not a critical factor implicated on hematopoietic differentiation.

5. Concluding remarks

The present study has shown that some neurotransmitters such as GABA and glutamate have interactions with most of the transcription factors and receptors involved in the hematopoietic stem cells differentiation in the adult Nile tilapia. Sub-basal increases of GABA, not greater than 0.1% above the baseline, can accelerate the differentiation of these cells during primordial, early and late hematopoiesis. However, their effects depend on a delicate balance of these interactions, particularly in the case of late hematopoiesis.

Declaration of interests

The authors declare no conflict of interest.

CRediT authorship contribution statement

Armando Vega-López: Writing - original draft. **Nataraj S. Pagadala:** Writing - original draft. **Minerva Nájera-Martínez:** Writing - original draft. **Elba Reyes-Maldonado:** Writing - original draft.

Acknowledgements

This study was supported by Instituto Politécnico Nacional SIP code 20170766 and SIP code 20180917. B.P. López-Tapia is graduate MSc student & R.L. Madera-Sandoval is graduate DSc. student which receive scholarship from CONACyT and BEIFI-IPN. A. Vega-López & E. Reyes-Maldonado are fellow of Estímulos al Desempeño en Investigación and Comisión y Fomento de Actividades Académicas (Instituto Politécnico Nacional); Vega-López, Nájera-Martínez & Reyes-Maldonado are fellow of Sistema Nacional de Investigadores (SNI, CONACyT, México).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.08.032.

References

[1] V.M. Valdespino-Gómez, P.M. Valdespino-Castillo, V.E. Valdespino-Castillo, [Cell signaling pathways interaction in cellular proliferation: potential target for therapeutic interventionism], Cir. Cir. 83 (2) (2015) 165–174 https://doi:10.1016/j.circir.2015.04.015.

[2] C.C. Capen, Toxic response of the endocrine system, Chapter 21, in: C.D. Klaassen (Ed.), Cassarett and Doull's Toxicology the Basic Science of Poisons, seventh ed., McGraw-Hill, Columbus, OH, 2008, pp. 807–879.

[3] J.M. Cerdá-Reverter, F. Canosa, Neuroendocrine systems of the fish brain, in: N.J. Bernier, G. Van Der Kraak, A.P. Farrell, C.J. Brauner (Eds.), Fish Neuroendocrinology. Fish Physiology Volume 28 Chapter, Neuroendocrine Systems of the Fish Brain Publisher, 2009, pp. 3–74 https://doi.org/10.1016/S1546-5098(09)28001-0.

[4] I.J. Clarke, Hypothalamus as an endocrine organ, Comp. Physiol. 5 (1) (2015) 217–253 https://doi:10.1002/cphy.c140019.

[5] D. Norris, J. Carr, D. Norris, J. Carr (Eds.), The Hypothalamus-Pituitary System in Non-mammalian Vertebrates, fifth ed., Vertebrate Endocrinology, Elsevier Inc, Oxford, UK, 2007, pp. 151–205.

[6] L.J. Jara, C. Navarro, G. Medina, O. Vera-Lastra, F. Blanco, Immune-neuroendocrine interactions and autoimmune diseases, Clin. Dev. Immunol. 13 (2–4) (2006) 109–123 https://doi:10.1080/17402520600877059.

[7] G. Nardocci, C. Navarro, P.P. Cortés, M. Imarai, M. Montoya, B. Valenzuela, P. Jara, C. Acuña-Castillo, R. Fernández R, Neuroendocrine mechanisms for immune system regulation during stress in fish, Fish Shellfish Immunol. 40 (2) (2014) 531–538 https://doi.org/10.1016/j.fsi.2014.08.001.

[8] B.M.L. Verburg-van Kemenade, N. Cohen, M. Chadzinska, Neuroendocrine-immune interaction: evolutionarily conserved mechanisms that maintain allostasis in an ever-changing environment, Dev. Comp. Immunol. 66 (2017) 2–23 https://doi:10.1016/j.dci.2016.05.015.

[9] A. Barragan, J.M. Weidner, Z. Jin, E.R. Korpi, B. Birnir, GABAergic signalling in the immune system, Acta Physiol. 213 (4) (2015) 819–827 https://doi:10.1111/apha.12467.

[10] G.J. Prud'homme, Y. Glinka, Q. Wang, Immunological GABAergic interactions and therapeutic applications in autoimmune diseases, Autoimmun. Rev. 14 (11) (2015) 1048–1056 https://doi:10.1016/j.autrev.2015.07.011.

[11] U. Andersson, K.J. Tracey, Reflex principles of immunological homeostasis, Annu. Rev. Immunol. 30 (2012) 313–335 https://doi:10.1146/annurev-immunol-020711-075015.

[12] J. Ordovas-Montanes, S. Rakoff-Nahoum, S. Huang, L. Riol-Blanco, O. Barreiro, U. H von Andrian, The regulation of immunological processes by peripheral neurons in homeostasis and disease, Trends Immunol. 36 (10) (2015) 578–604 https://doi:10.1016/j.it.2015.08.007.

[13] A. Al-Sharea, M.K.S. Lee, A. Whillas, M.C. Flynn, J. Chin-Dusting, A.J. Murphy, Nicotinic acetylcholine receptor alpha 7 stimulation dampens splenic myelopoiesis and inhibits atherogenesis in Apoe-/- mice, Atherosclerosis 265 (2017) 47–53 https://doi:10.1016/j.atherosclerosis.2017.08.010.

[14] A. López-Requena, B. Boonen, L. Van Gerven, P.W. Hellings, Y.A. Alpizar, K. Talavera. Roles of neuronal TRP channels in neuroimmune interactions, chapter 15. in: T.L.R. Emir (Ed), Neurobiology of TRP Channels second ed., CRC Press/Taylor & Francis, Boca Raton (FL), Frontiers in Neuroscience. https://www.ncbi.nlm.nih.gov/books/NBK476103/.

[15] L. Teng, Y.B. Tang, F. Sun, S.M. An, C. Zhang, X.J. Yang, H.Y. Lv, Q. Lu, Y.Y. Cui, J.J. Hu, L. Zhu, H.Z. Chen, Non-neuronal release of gamma-aminobutyric acid by embryonic pluripotent stem cells, Stem Cells Dev. 22 (22) (2013) 2944–2953 https://doi:10.1089/scd.2013.0243.

[16] H. Igarashi, S.C. Gregory, T. Yokota, N. Sakaguchi, P.W. Kincade, Transcription from the RAG1 locus marks the earliest lymphocyte progenitors in bone marrow, Immunity 17 (2) (2002) 117–130 https://doi.org/10.1016/S1074-7613(02)00366-7.

[17] R.S. Welner, P.W. Kincade, R. Pelayo, Early lymphopoiesis in adult bone marrow, Inmunologia 26 (3) (2007) 121–171 https://doi.org/10.1016/S0213-9626(07)70083-5.

[18] L. Ding, S.J. Morrison, Haematopoietic stem cells and early lymphoid progenitors occupy distinct bone marrow niches, Nature 495 (7440) (2013) 231–235.

[19] Y. Huang, J. Cai, J.F. Tang, H.Y. Zhang, Z.W. Wang, J.C. Jian, Z.H. Wu, Y.S. Lu, Identification and expression analysis of terminal deoxynucleotidyl transferase in humphead snapper *Lutjanus sanguineus*, J. Fish Biol. 90 (5) (2017) 2194–2199 https://doi:10.1111/jfb.13259.

[20] M.Y. Khuhawar, A.D. Rajper, Liquid chromatographic determination of gamma-aminobutyric acid in cerebrospinal fluid using 2-hydroxynaphthaldehyde as derivatizing reagent, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 788 (2) (2003) 413–418 https://doi.org/10.1016/S1570-0232(03)00062-X.

[21] SAGARPA. Norma Oficial Mexicana NOM-062-ZOO-1999 Especificaciones técnicas para la producción, cuidado y el uso de animales de laboratorio, Diario Oficial de la Federación, 2001, p. 22 de agosto de 2001 http://www.fmvz.unam.mx/fmvz/principal/archivos/062ZOO.PDF.

[22] R.L. Madera-Sandoval, E. Reyes-Maldonado, R. Dzul-Caamal, E. Gallegos-Rangel, M.L. Domínguez-López, E. García-Latorre, A. Vega-López, Fat-associated lymphoid cluster in *Cyprinus carpio*: characterisation and its relation with peritoneal haemangiosarcoma, Fish Shellfish Immunol. 44 (2) (2015) 633–641 https://doi.org/10.1016/j.fsi.2015.03.019.

[23] K. Deng, M. Pan, J. Liu, M. Yang, Z. Gu, Y. Zhang, G. Liu, D. Liu, W. Zhang, K. Mai, Chronic stress of high dietary carbohydrate level causes inflammation and influences glucose transport through SOCS3 in Japanese flounder *Paralichthys olivaceus*, Sci. Rep. 8 (1) (2018) 7415 https://doi.org/10.1038/s41598-018-25412-w.

[24] H. Shibata, Y. Hanazono, N. Ageyama, T. Nagashima, Y. Ueda, M. Hasegawa, K. Ozawa, Y. Yoshikawa, K. Terao, Collection and analysis of hematopoietic progenitor cells from cynomolgus macaques (*Macaca fascicularis*): assessment of cross-reacting monoclonal antibodies, Am. J. Primatol. 61 (1) (2003) 3–12 https://doi.org/10.1002/ajp.10104.

[25] N.A. Wong, Z. Melegh, Antigen retrieval and primary antibody type affect sensitivity but not specificity of CD117 immunohistochemistry, Histopathology 54 (5) (2009) 529–538 https://doi.org/10.1111/j.1365-2559.2009.03272.x.

[26] R.P. Panda, H.K. Barman, C. Mohapatra, Isolation of enriched carp spermatogonial stem cells from *Labeo rohita* testis for *in vitro* propagation, Theriogenology 76 (2) (2011) 241–251 https://doi.org/10.1016/j.theriogenology.2011.01.031.

[27] D. Traver, B.H. Paw, K.D. Poss, W.T. Penberthy, S. Lin, L. Zon LI, Transplantation and in vivo imaging of multilineage engraftment in zebrafish bloodless mutants, Nat. Immunol. 4 (2003) 1238–1246.

[28] A. Sali, T.L. Blundell, Comparative protein modelling by satisfaction of spatial restraints, J. Mol. Biol. 234 (1993) 779–815 https://doi.org/10.1006/jmbi.1993.1626.

[29] R.A. Laskowski, M.W. MacArthur, D.S. Moss, J.M. Thornton, PROCHECK: a program to check the stereochemical quality of protein structures, J. Appl. Crystallogr. 26 (1993) 283–291.

[30] C. Colovos, T.O. Yeates, Verification of protein structures: patterns of nonbonded atomic interactions, Protein Sci. 2 (9) (1993) 1511–1519 https://doi.org/10.1002/pro.5560020916.

[31] G. Vriend, Parameter relation rows: a query system for protein structure function relationships, Protein Eng. 4 (1990) 221–223 https://doi.org/10.1093/protein/4.2.221.

[32] R.W. Hooft, G. Vriend, C. Sander, E.E. Abola, Errors in protein structures, Nature 381 (1996) 272-272 https://doi.org/10.1038/381272a0.

[33] D. Eisenberg, R. Lüthy, J.U. Bowie, VERIFY3D: assessment of protein models with three-dimensional profiles, Methods Enzymol. 277 (1997) 396–404 https://doi.org/10.1016/S0076-6879(97)77022-8.

[34] H. Edelsbrunner, M. Facello, Fu Ping, J. Liang, Measuring proteins and voids in proteins, Proceedings of the 28th Annual Hawaii International Conference on Systems Science, 1995, pp. 256–264 https://doi.org/10.1109/HICSS.1995.375331.

[35] N.M. Rowley, K.K. Madsen, A. Schousboe, H. Steve White, Glutamate and GABA synthesis, release, transport and metabolism as targets for seizure control, Neurochem. Int. 61 (4) (2012) 546–558 https://doi.org/10.1016/j.neuint.2012.02.013.

[36] Z. Hevessy, B. Nagy Jr., F. Kiss, A. Kiss, J. Kappelmayer, Mean fluorescence intensity rate is a useful marker in the detection of paroxysmal nocturnal hemoglobinuria clones, Clin. Chem. Lab. Med. 43 (9) (2005) 919–923 https://doi:10.1515/CCLM.2005.157.

[37] K. El Omari, S.J. Hoosdally, K. Tuladhar, D. Karia, E. Hall-Ponselé, O. Platonova, P. Vyas, R. Patient, C. Porcher, E.J. Mancini, Structural basis for LMO2-driven recruitment of the SCL:E47bHLH heterodimer to hematopoietic-specific transcriptional targets, Cell Rep. 4 (1) (2013) 135–147 https://doi:10.1016/j.celrep.2013.06.008.

[38] S. Salmen, N. Silva-Gutierrez, R. Bahsas-Zaky, G. Terán-Angel, L. Barboza, K. Padrón, L. Berrueta, D. Olávez, E. Solórzano, A. Calderón, J.C. Valencia-Molina, M. Soto-Parra, I. Volcanes, E.A. Paredes, M. Rondon, Células progenitoras pluripotenciales: características y compartimientos especializados de residencia, Avan. Biomed. 2 (1) (2013) 26–38.

[39] G.M. Crane, E. Jeffery, S.J. Morrison, Adult haematopoietic stem cell niches, Nat. Rev. Immunol. 17 (9) (2017) 573–590 https://doi:10.1038/nri.2017.53.

[40] H.K. Kong, J.H. Park, Characterization and function of human Ly-6/uPAR molecules, BMB Rep. 45 (11) (2012) 595–603 https://doi:10.5483/BMBRep.2012.45.11.210.

[41] B. Wu, E.Y. Chien, C.D. Mol, G. Fenalti, W. Liu, V. Katritch, R. Abagyan, A. Brooun, P. Wells, F.C. Bi, D.J. Hamel, P. Kuhn, T.M. Handel, V. Cherezov, R.C. Stevens, Structures of the CXCR4 chemokine GPCR with small-molecule and cyclic peptide antagonists, Science 330 (6007) (2010) 1066–1071 https://doi.org/10.1126/science.1194396.

[42] J. Griffith, J. Black, C. Faerman, L. Swenson, M. Wynn, F. Lu, J. Lippke, K. Saxena, The structural basis for autoinhibition of FLT3 by the juxtamembrane domain, Mol. Cell 13 (2) (2004) 169–178 https://doi.org/10.1016/S1097-2765(03)00505-7.

[43] T. Naoe, H. Kiyoi, Normal and oncogenic FLT3, CMLS Cell. Mol. Life Sci. 61 (23) (2004) 2932–2938 https://doi.org/10.1007/s00018-004-4274-x.

[44] A. Ivanovs, S. Rybtsov, E.S. Ng, E.G. Stanley, A.G. Elefanty, A. Medvinsky, Human haematopoietic stem cell development: from the embryo to the dish, Development 144 (13) (2017) 2323–2337 https://doi:10.1242/dev.134866.

[45] J.H. Wang, R.B. Pepinsky, T. Stehle, J.H. Liu, M. Karpusas, B. Browning, L. Osborn, The crystal structure of an N-terminal two-domain fragment of vascular cell adhesion molecule 1 (VCAM-1): a cyclic peptide based on the domain 1 C-D loop can inhibit VCAM-1-alpha 4 integrin interaction, Proc. Natl. Acad. Sci. U.S.A. 92 (12) (1995) 5714–5718 https://doi.org/10.1073/pnas.92.12.5714.

[46] R.A. Oostendorp, P. Dörmer, VLA-4-mediated interactions between normal human hematopoietic progenitors and stromal cells, Leuk. Lymphoma 24 (5–6) (1997) 423–435 https://doi.org/10.3109/10428199709055581.

[47] M.D. Feese, T. Tamada, Y. Kato, Y. Maeda, M. Hirose, Y. Matsukura, H. Shigematsu, T. Muto, A. Matsumoto, H. Watarai, K. Ogami, T. Tahara, T. Kato, H. Miyazaki, R. Kuroki, Structure of the receptor-binding domain of human thrombopoietin determined by complexation with a neutralizing antibody fragment, Proc. Natl. Acad. Sci. U.S.A. 101 (7) (2004) 1816–1821 https://doi.org/10.1073/pnas.0308530100.

[48] J.N. Ihle, F.W. Quelle, O. Miura, Signal transduction through the receptor for erythropoietin, Semin. Immunol. 5 (5) (1993) 375–389 https://doi.org/10.1006/smim.1993.1043.

[49] S.J. McGee, A.M. Havens, Y. Shiozawa, Y. Jung, R.S. Taichman, Effects of erythropoietin on the bone microenvironment, Growth Factors 30 (1) (2012) 22–28 https://doi:10.3109/08977194.2011.637034.

[50] R. Ferrao, P.F. Lupardus, The janus kinase (JAK) FERM and SH2 domains: bringing specificity to JAK-receptor interactions, Front. Endocrinol. 8 (2017) 71 eCollection 2017 https://doi:10.3389/fendo.2017.00071.

[51] Y. Chen, P. Haviernik, K.D. Bunting, Y.C. Yang, Cited2 is required for normal hematopoiesis in the murine fetal liver, Blood 110 (8) (2012) 2889–2898 https://doi.org/10.1182/blood-2007-01-066316.

[52] C.R. Escalante, A.L. Brass, J.M. Pongubala, E. Shatova, L. Shen, H. Singh, A.K. Aggarwal, Crystal structure of PU.1/IRF-4/DNA ternary complex, Mol. Cell 10 (5) (2002) 1097–1105 https://doi.org/10.1016/S1097-2765(02)00703-7.

[53] T. Yashiro, K. Kasakura, Y. Oda, N. Kitamura, A. Inoue, S. Nakamura, H. Yokoyama, J. Fukuyama, M. Hara, H. Ogawa, K. Okumura, M. Nishiyama, C. Nishiyama, The hematopoietic cell-specific transcription factor PU.1 is critical for expression of CD11c, Int. Immunol. 29 (2) (2017) 87–94 https://doi:10.1093/intimm/dxx009.

[54] S.H.M. Pang, C.A. de Graaf, D.J. Hilton, N.D. Huntington, S. Carotta, L. Wu, S.L. Nutt, PU, 1 is required for the developmental progression of multipotent progenitors to common lymphoid progenitors, Front. Immunol. 9 (2018) 1264 eCollection 2018 https://doi:10.3389/fimmu.2018.01264.

[55] E.W. Ades, R.K. Zwerner, R.T. Acton, C.M. Balch, Isolation and partial characterization of the human homologue of Thy-1, J. Exp. Med. 151 (2) (1980) 400–406 https://doi:10.1084/jem.151.2.400.

[56] B.A. Miller, J.M. Lipton, Thy-1 antigen in murine hematopoietic progenitor differentiation, Prog. Clin. Biol. Res. 134 (1983) 389–397 https://www.ncbi.nlm.nih.gov/pubmed/6141570.

[57] F.J. Sharom, M.T. Lehto, Glycosylphosphatidylinositol-anchored proteins: structure, function, and cleavage by phosphatidylinositol-specific phospholipase C, Biochem. Cell Biol. 80 (5) (2002) 535–549 https://doi.org/10.1139/o02-146.

[58] R. Ceredig, A.G. Rolink, The key role of IL-7 in lymphopoiesis, Semin. Immunol. 24 (3) (2012) 159–164 https://doi.org/10.1016/j.smim.2012.02.004.

[59] C.A. McElroy, J.A. Dohm, S.T. Walsh, Structural and biophysical studies of the human IL-7/IL-7Ralpha complex, Structure 17 (1) (2009) 54–65 https://doi.org/10.1016/j.str.2008.10.019.

[60] Z. Keren, D. Melamed, Antigen receptor signaling competence and the determination of B cell fate in B-lymphopoiesis, Histol. Histopathol. 20 (1) (2005) 187–196 https://doi.org/10.14670/HH-20.187.

[61] S.J. Keppler, F. Gasparrini, M. Burbage, S. Aggarwal, B. Frederico, R.S. Geha, M. Way, A. Bruckbauer, F.D. Batista, Wiskott-aldrich syndrome interacting protein deficiency uncovers the role of the Co-receptor CD19 as a generic hub for PI3 kinase signaling in B cells, Immunity 43 (4) (2015) 660–673 https://doi.org/10.1016/j.immuni.2015.09.004.

[62] B.M. Stadtmueller, K.E. Huey-Tubman, C.J. López, Z. Yang, W.L. Hubbell, P.J. Bjorkman, The structure and dynamics of secretory component and its interactions with polymeric immunoglobulins, eLife 4 (2016) 5 pii: e10640 https://doi.org/10.7554/eLife.10640.001.

[63] C. Schubert, C. Schalk-Hihi, G.T. Struble, H.C. Ma, I.P. Petrounia, B. Brandt, I.C. Deckman, R.J. Patch, M.R. Player, J.C. Spurlino, B.A. Springer, Crystal structure of the tyrosine kinase domain of colony-stimulating factor-1 receptor (cFMS) in complex with two inhibitors, J. Biol. Chem. 282 (6) (2007) 4094–4101 https://doi.org/10.1074/jbc.M608183200.

[64] X. Chen, H. Liu, P.J. Focia, A.H. Shim, X. He, Structure of macrophage colony

stimulating factor bound to FMS: diverse signaling assemblies of class III receptor tyrosine kinases, Proc. Natl. Acad. Sci. U.S.A. 105 (47) (2008) 18267–18272 https://doi.org/10.1073/pnas.0807762105.

[65] M. Endele, D. Loeffler, K.D. Kokkaliaris, O. Hilsenbeck, S. Skylaki, P.S. Hoppe, A. Schambach, E.R. Stanley, T. Schroeder, CSF-1-induced Src signaling can instruct monocytic lineage choice, Blood 129 (12) (2017) 1691–1701 https://doi.org/10.1182/blood-2016-05-714329.

[66] E.H. Abdel-Aziz, S.B.S. Abdu, T.E. Ali, H.F. Fouad, Haemopoiesis in the head kidney of tilapia, *Oreochromis niloticus* (Teleostei: cichlidae): a morphological (optical and ultrastructural) study, Fish Physiol. Biochem. 36 (3) (2010) 323–336 https://doi.org/10.1007/s10695-008-9297-z.

[67] J.D. Hansen, A.G. Zapata, Lymphocyte development in fish and amphibians, Immunol. Rev. 166 (1) (1998) 199–220 https://doi.org/10.1111/j.1600-065x.1998.tb01264.x.

[68] N.S. Trede, A. Zapata, L.I. Zon, Fishing for lymphoid genes, Trends Immunol. 22 (6) (2001) 302–307 https://doi.org/10.1016/S1471-4906(01)01939-1.

[69] A.J. Bloor, M.J. Sánchez, A.R. Green, B. Göttgens, The role of the stem cell leukemia (SCL) gene in hematopoietic and endothelial lineage specification, J. Hematother. Stem Cell Res. 11 (2) (2002) 195–206 https://doi.org/10.1089/152581602753658402.

[70] S. Rojas-Sutterlin, E. Lecuyer, T. Hoang, Kit and Scl regulation of hematopoietic stem cells, Curr. Opin. Hematol. 21 (4) (2014) 256–264 https://doi.org/10.1097/MOH.0000000000000052.

[71] J.Y. Shin, W. Hu, M. Naramura, C.Y. Park, High c-Kit expression identifies hematopoietic stem cells with impaired self-renewal and megakaryocytic bias, J. Exp. Med. 211 (2) (2014) 217–231 https://doi.org/10.1084/jem.20131128.

[72] E. Khodadi, S. Shahrabi, M. Shahjahani, S. Azandeh, N. Saki, Role of stem cell factor in the placental niche, Cell Tissue Res. 366 (3) (2016) 523–531 https://doi.org/10.1007/s00441-016-2429-3.

[73] T.M. Schlaeger, A. Schuh, S. Flitter, A. Fisher, H. Mikkola, S.H. Orkin, P. Vyas, C. Porcher, Decoding hematopoietic specificity in the helix-loop-helix domain of the transcription factor SCL/Tal-1, Mol. Cell. Biol. 24 (17) (2004) 7491–7502 https://doi.org/10.1128/MCB.24.17.7491-7502.2004.

[74] S.B. Bradfute, T.A. Graubert, M.A. Goodell, Roles of Sca-1 in hematopoietic stem/progenitor cell function, Exp. Hematol. 33 (2005) 836–843 https://doi.org/10.1016/j.exphem.2005.04.001.

[75] H.S. Bernstein, T. Samad, S. Cholsiripunlert, S. Khalifian, W. Gong, C. Ritner, J. Aurigui, V. Ling, K.J. Wilschut, S. Bennett, J. Hoffman, P. Oishi, Stem cell antigen-1 in skeletal muscle function, PLoS Curr. 15 (5) (2013), https://doi.org/10.1371/currents.md.411a8332d61e22725e6937b97e6d0ef8.

[76] J. Ciriza, M.E. García-Ojeda, Expression of migration-related genes is progressively upregulated in murine Lineage-Sca-1 + c-Kit + population from the fetal to adult stages of development, Stem Cell Res. Ther. 1 (2) (2010) 14 https://doi.org/10.1186/scrt14.

[77] B. Wu, E.Y. Chien, C.D. Mol, G. Fenalti, W. Liu, V. Katritch, R. Abagyan, A. Brooun, P. Wells, F.C. Bi, D.J. Hamel, P. Kuhn, T.M. Handel, V. Cherezov, R.C. Stevens, Structures of the CXCR4 chemokine GPCR with small-molecule and cyclic peptide antagonists, Science 330 (6007) (2010) 1066–1071 https://doi.org/10.1126/science.1194396.

[78] N. An, Y.W. Lin, S. Mahajan, J.N. Kellner, Y. Wang, Z. Li, A.S. Kraft, Y. Kang, Pim1 serine/threonine kinase regulates the number and functions of murine hematopoietic stem cells, Stem Cells 31 (6) (2013) 1202–1212 https://doi.org/10.1002/stem.1369.

[79] J. Liu, J. Zhao, J.F. Lee, A. Gartung, H. Jawadi, W. Zhang, D. Lominadze, M.J. Lee, 3-amino-4-(3-hexylphenylamino)-4-oxobutyl phosphonic acid (W146), a selective antagonist of sphingosine-1-phospahte receptor subtype 1, enhances AMD3100-stimulated mobilization of hematopoietic stem progenitor cells in animals, J. Biochem. Pharmacol. Res. 1 (4) (2013) 197–203 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4221244/.

[80] Y. Liu, H. Liu, C. Sauvey, L. Yao, E.D. Zarnowska, S.C. Zhang, Directed differentiation of forebrain GABA interneurons from human pluripotent stem cells, Nat. Protoc. 8 (9) (2013) 1670–1679 https://doi.org/10.1038/nprot.2013.106.

[81] B. Doron, M. Handu, P. Kurre, Concise review: adaptation of the bone marrow stroma in hematopoietic malignancies: current concepts and models, Stem Cells 36 (3) (2018) 304–312 https://doi.org/10.1002/stem.2761.

[82] A. Kayaba, A. Itoh-Nakadai, K. Niibe, M. Shirota, R. Funayama, A. Sugahara-Tobinai, Y.L. Wong, M. Inui, K. Nakayama, T. Takai, Bone marrow PDGFRα + Sca-1 + -enriched mesenchymal stem cells support survival of and antibody production by plasma cells in vitro through IL-6, Int. Immunol. 30 (6) (2018) 241–253 https://doi.org/10.1093/intimm/dxy018.

[83] K. Wang, X. Zhao, C. Kuang, D. Qian, H. Wang, H. Jiang, M. Deng, L. Huang, Overexpression of SDF-1α enhanced migration and engraftment of cardiac stem cells and reduced infarcted size via CXCR4/PI3K pathway, PLoS One 7 (9) (2012) e43922https://doi.org/10.1371/journal.pone.0043922.

[84] J.H. Choi, M.P. Nguyen, S.Y. Jung, S.M. Kwon, J.G. Jee, J.S. Bae, S. Lee, M.Y. Lee, Y.M. Lee, Inhibitory effect of glyceollins on vasculogenesis through suppression of endothelial progenitor cell function, Mol. Nutr. Food Res. 57 (10) (2013) 1762–1771 https://doi.org/10.1002/mnfr.201200826.

[85] P. Krishnamurthy, M. Thal, S. Verma, E. Hoxha, E. Lambers, V. Ramirez, G. Qin, D. Losordo, R. Kishore, Interleukin-10 deficiency impairs bone marrow-derived endothelial progenitor cell survival and function in ischemic myocardium, Circ. Res. 109 (11) (2011) 1280–1289 https://doi.org/10.1161/CIRCRESAHA.111.248369.

[86] K. Limbkar, A. Dhenge, D.D. Jadhav, H.V. Thulasiram, V. KalCIe, L. Limaye, Data on the effect of oral feeding of Arachidonic acid or Docosahexanoic acid on haematopoiesis in mice, Data Brief 14 (2017) 551–557 https://doi.org/10.1016/j.dib.2017.08.009.

[87] V. Mignotte, I. Vigon, E. Boucher de Crèvecoeur, P.H. Roméo, V. Lemarchandel, S. Chrétien, Structure and transcription of the human c-mpl gene (MPL), Genomics 20 (1) (1994) 5–12 https://doi.org/10.1006/geno.1994.1120.

[88] Y. Miyakawa, A. Oda, B.J. Druker, T. Kato, H. Miyazaki, M. Handa, Y. Ikeda, Recombinant thrombopoietin induces rapid protein tyrosine phosphorylation of Janus kinase 2 and Shc in human blood platelets, Blood 86 (1) (1995) 23–27 https://doi.org/10.1016/S0887-7963(96)80140-4.

[89] M.C. Rouyez, C. Boucheron, S. Gisselbrecht, I. Dusanter-Fourt, F. Porteu, Control of thrombopoietin-induced megakaryocytic differentiation by the mitogen-activated protein kinase pathway, Mol. Cell. Biol. 17 (9) (1997) 4991–5000 https://doi.org/10.1128/MCB.17.9.4991.

[90] I. Matsumura, K. Nakajima, H. Wakao, S. Hattori, K. Hashimoto, H. Sugahara, T. Kato, H. Miyazaki, T. Hirano, Y. Kanakura, Involvement of prolonged ras activation in thrombopoietin-induced megakaryocytic differentiation of a human factor-dependent hematopoietic cell line, Mol. Cell. Biol. 18 (7) (1998) 4282–4290 https://doi.org/10.1128/MCB.18.7.4282.

[91] W. Tong, R. Sulahian, A.W. Gross, N. Hendon, H.F. Lodish, L.J. Huang, The membrane-proximal region of the thrombopoietin receptor confers its high surface expression by JAK2-dependent and -independent mechanisms, J. Biol. Chem. 281 (50) (2006) 38930–38940 https://doi.org/10.1074/jbc.M607524200.

[92] R.I. Albu, S.N. Constantinescu, Extracellular domain N-glycosylation controls human thrombopoietin receptor cell surface levels, Front. Endocrinol. 2 (2011) 71 2011 https://doi.org/10.3389/fendo.2011.00071.

[93] F. Guarnieri, Analysis of the asymmetry of activated EPO receptor enables designing small molecule agonists, Vitam. Horm. 105 (2017) 19–37 https://doi.org/10.1016/bs.vh.2017.03.004.

[94] J. Shi, B. Yuan, W. Hu, H. Lodish, JAK2 V617F stimulates proliferation of erythropoietin-dependent erythroid progenitors and delays their differentiation by activating Stat1 and other nonerythroid signaling pathways, Exp. Hematol. 44 (11) (2016) 1044–1058 e5 https://doi.org/10.1016/j.exphem.2016.07.010.

[95] H.E. Broxmeyer Erythropoietin, Multiple targets, actions, and modifying influences for biological and clinical consideration, Exp. Med. 210 (2) (2013) 205–208 https://doi.org/10.1084/jem.20122760.

[96] P. Sahai-Hernandez, D. Traver, Intimacy of the niche: perivascular remodeling cuddles incoming HSCs, Cell Stem Cell 16 (2) (2015) 109–110 https://doi:10.1016/j.stem.2015.01.011.

[97] J.R. Perlin, A.L. Robertson, L.I. Zon, Efforts to enhance blood stem cell engraftment: recent insights from zebrafish hematopoiesis, J. Exp. Med. 214 (10) (2017) 2817–2827 2017 https://doi:10.1084/jem.20171069.

[98] P. Charbord, C. Pouget, H. Binder, F. Dumont, G. Stik, P. Levy, F. Allain, C. Marchal C, J. Richter, B. Uzan, F. Pflumio, F. Letourneur, H. Wirth, E. Dzierzak, D. Traver, T. Jaffredo, C. Durand, A systems biology approach for defining the molecular framework of the hematopoietic stem cell niche, Cell Stem Cell 15 (3) (2014) 376–391 https://doi:10.1016/j.stem.2014.06.005.

[99] Y. Lee, J.E. Manegold, A.D. Kim, C. Pouget, D.L. Stachura, W.K. Clements, D. Traver, FGF signalling specifies haematopoietic stem cells through its regulation of somitic Notch signalling, Nat. Commun. 5 (2014) 5583 https://doi:10.1038/ncomms6583.

[100] C. Pouget, T. Peterkin, F.C. Simões, Y. Lee, D. Traver, R. Patient, FGF signalling restricts haematopoietic stem cell specification via modulation of the BMP pathway, Nat. Commun. 5 (2014) 5588 https://doi:10.1038/ncomms6588.

[101] E. Dzierzak, N.A. Speck, Of lineage and legacy: the development of mammalian hematopoietic stem cells, Nat. Immunol. 9 (2) (2008) 129–136 https://doi:10.1038/ni1560.

[102] B.W. Blaser, J.L. Moore, E.J. Hagedorn, B. Li, R. Riquelme, A. Lichtig, S. Yang, Y. Zhou, O.J. Tamplin, V. Binder, L.I. Zon, CXCR1 remodels the vascular niche to promote hematopoietic stem and progenitor cell engraftment, J. Exp. Med. 214 (4) (2017) 1011–1027 https://doi:10.1084/jem.20161616.

[103] P.J. Simmons, J.P. Levesque, D.N. Haylock, Mucin-like molecules as modulators of the survival and proliferation of primitive hematopoietic cells, Ann. N. Y. Acad. Sci. 938 (2001) 196–206 discussion 206-7.