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Stem cells to decipher the physiological roles of 5-HT_{2B} receptor signaling

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Abstract The serotonergic 2B receptor (5-HT_{2B}R), expressed in the central nervous system and at the periphery, contributes to the regulation of several physiological processes such as cardio-vascular, pulmonary, bone and neuronal functions. Due to its multifaceted roles, signaling pathways and cell targets controlled by the 5-HT_{2B}R are still the subject of intensive research. Here, we review how exploiting the dynamics of differentiation of the 1C11 neuronal stem cell line allows to identify several 5-HT_{2B}R-coupled signaling pathways and targets, among which the serotonin transporter, involved in the homeostasis of serotonergic neurons and their adaptive response to selective serotonin-reuptake inhibitors (SSRIs) like fluoxetine (Prozac[®]). In addition mesoblastic stem cell lines permit to show that along the osteogenic differentiation, the 5-HT_{2B}R controls the activity of the tissue-non-specific alkaline phosphatase, a major enzyme for bone formation. Finally the 5-HT_{2B}R expressed by pulpal stem cells takes part to natural tooth repair upon 5-HT release by injury-activated platelets.

1 Introduction

In the central nervous system and at the periphery, the bioamine serotonin (5-hydroxytryptamine; 5-HT) is involved in a myriad of physiological processes including sleep, mood, memory, cognition, appetite, as well as cardiovascular, digestive and endocrine functions [1, 2]. In the brain, the source of 5-HT is the raphe nuclei and, at the periphery, 5-HT mainly originates from the intestinal enterochromaffin cells. 5-HT exerts its diverse roles through a constellation of serotonergic receptors. Since the 1970s, fifteen serotonergic receptor subtypes (5-HTR) have been evidenced and classified into seven families according to structural and pharmacological criteria as well as coupled signal transduction pathways (5-HT₁₋₇R) [3]. While the 5-HT₃R is a ionotropic receptor, all other 5-HT receptors are metabotropic receptors coupled to G proteins (GPCR) [4]. The intensity and duration of 5-HT receptor signaling depends on the reuptake of 5-HT exerted by the 5-HT membrane transporter, the SERT (serotonin reuptake transporter), a well-known target for selective serotonin-reuptake inhibitors (SSRIs) antidepressants such as fluoxetine (Prozac[®]) and paroxetine.

The 5-HT_{2B} receptor (5-HT_{2B}R) belongs to the 5-HT₂ receptor subfamily, which also includes the 5-HT_{2A}R and the 5-HT_{2C}R. The gene encoding the 5-HT_{2B}R was cloned from the mouse brain, rat stomach fundus, human liver and placenta between 1992 and 1994 [5-10]. The human 5-HT_{2B}R gene is located on chromosome 2 (2q36.3-2q37.1) [11]. During embryonic development, the 5-HT_{2B}R mRNA is detected in the mouse embryo as soon as day 8.5-9 post coitum in the heart primordia, the neural tube before its closure and in the first branchial arch at the origin of craniofacial derivatives such as maxilla, mandible and teeth [5, 12]. Of note, at this stage, the 5-HT_{2A}R and 5-HT_{2C}R mRNAs are also expressed at low levels [12]. As maternal 5-HT plays crucial trophic functions at early steps of murine development [13], this

specific pattern of 5-HT_{2B}R expression just after the beginning of gastrulation indicates a key role of this receptor during the embryonic morphogenesis of the cardiovascular and craniofacial structures as well as the nervous system. This is supported by the heart and brain malformations observed in mouse embryos exposed to the 5-HT_{2B}R inverse agonist ritanserin and in 5-HT_{2B}R knockout mice. Ritanserin-treated embryos exhibit an underdevelopment of the forebrain, hindbrain and pharyngeal arches, heart defects and closure failure of the neural tube. This led to the assumption that the 5-HT_{2B}R plays a fundamental role in neural crest migration, cell proliferation and/or survival [12, 14]. Accordingly, depletion of 5-HT_{2B}R in mice leads to death of two third of the mutant population during the gestation or at birth due to severe cardiac defects [15].

In adult rodents, the 5-HT_{2B}R is mainly expressed at the periphery in the cardiovascular and gastrointestinal systems, and is also detectable at lower levels in the brain, notably in the cerebellum, hypothalamus, hippocampus, amygdala and raphe nuclei [6, 16-18]. During the past 25 years, the use of pharmacological drugs and the characterization of 5-HT_{2B}R^{-/-} mice provided evidence that the 5-HT_{2B}R is involved in the control of a wide range of physiological functions, including cardiac, vascular, pulmonary, bone, gastrointestinal and cerebral functions (for reviews see [19-21] and references therein). Deregulation of 5-HT_{2B}R expression and signaling is associated with various pathological conditions such as fibrosis, pulmonary arterial hypertension and cancer [20, 22-25].

Over the last decades, the identification of the signaling pathways mobilized by the 5-HT_{2B}R and the downstream effectors led to a better understanding of the pathophysiological role of the 5-HT_{2B}R. The present review emphasizes the contribution of neuronal and mesodermal stem cell lines that endogenously express the 5-HT_{2B}R to grasp the signaling network driven by the 5-HT_{2B}R to ensure the onset of neuronal and/or bone functions and therefore homeostasis of bioaminergic neurons and mineralized tissues.

2 The 1C11 neuronal stem cell line to dissect the role of 5-HT_{2B}Rs in bioaminergic neurons

2.1 5-HT_{2B}Rs are involved in the neuronal differentiation program of 1C11 stem cells

Isolated in 1990 from murine multipotent cells, the 1C11 cell line behaves as a bipotential neuronal stem cell that has the intrinsic properties to differentiate upon appropriate induction into serotonergic (1C11^{5-HT}) and noradrenergic (1C11^{NE}) neuronal cells at a frequency of around 100% in a mutually exclusive manner (Fig. 1) [26-28].

Upon 4 days addition of dibutyryl cyclic AMP (dbcAMP) and cyclohexane carboxylic acid (CCA), there is a switch from the neuroepithelial precursor 1C11 cells to the 1C11^{5-HT} cells. The 1C11^{5-HT} neuronal cells have developed bipolar extensions, express neuron-associated markers (NCAM, synaptophysin,...) and acquired a complete serotonergic phenotype, *i.e.* the capacity to synthesize, store, catabolize and transport 5-HT [26, 28]. During the course of the serotonergic program, 1C11 cells acquires at definite times three 5-HT receptors (5-HT_{2B}R, 5-HT_{1B/1D}R and 5-HT_{2A}R) (Fig. 1) [29], whose expression was evidenced *in vivo* in serotonergic neurons of the raphe nuclei [30-33]. Two days after the addition of the inducers, the 5-HT_{2B}R (Kd 21,9 nM, 2500 receptors/cells) and 5-HT_{1B/1D}R (Kd 0.53 nM, 1200 receptors/cell) are functionally expressed and their density remains constant along the differentiation [29]. At day 4, concomitantly with the onset of the SERT expression, a functional 5-HT_{2A}R (Kd 0.85 nM, 400 receptors/cell) is induced. Of note, at day 2, 1C11 cells start to synthesize and catabolize 5-HT. The presence of the 5-HT_{2B}R and 5-HT_{1B/1D}R at this stage renders 1C11 stem cells competent to respond to 5-HT during their differentiation program. Thus, 5-HT_{2B}R and/or 5-HT_{1B/1D}R acting as autoreceptors contribute to the onset of a complete neuronal phenotype [28]. Treatment of differentiating 1C11^{5-HT} cells with ritanserin from day 2 reduces the intensity of neuronal functions measured at the end of the serotonergic program.

In presence of dbcAMP, CCA and DMSO, the serotonergic differentiation is blocked. 1C11 progenitor cells then convert in 12 days into noradrenergic 1C11^{NE} cells, which express a complete catecholaminergic phenotype *i.e.* the capacity to synthesize, store, catabolize and transport norepinephrine (NE) (Fig. 1) [28]. Along the noradrenergic differentiation, 1C11 cells implement a unique α_{1D} adrenoreceptor at day 8 (Kd 1.1 nM, 2200 receptors/cell) and NE transporter (NET) at day 12. Of note, blocking the α_{1D} adrenoreceptor with an antagonist at day 8 avoids the onset of NET at day 12, suggesting that the α_{1D} receptor is necessary for the completion of the noradrenergic phenotype. The α_{1D} adrenoreceptor in the noradrenergic program seems to play a role similar to the 5-HT_{2B}R along the serotonergic differentiation. The 1C11 neuronal stem cell line is thus a helpful paradigm to investigate the signaling and roles of bioaminergic receptors within an integrated serotonergic or noradrenergic context.

2.2 The onset of serotonergic functions mainly depends on post-transcriptional controls

In the 1C11 cell system, the transcriptional and translational control mechanisms that orchestrate the time-scheduled and effective implementation of neuronal functions during both serotonergic and noradrenergic programs remain elusive. *In vivo*, the differentiation of central 5-HT neurons depends on the transcription factors Nkx2-2, Lmx1b, Pet1 and Mash1 [34]. Strikingly, in the 1C11 cell line, the mRNAs encoding serotonergic and noradrenergic functions, such as neurotransmitter synthesis enzymes (tyrosine hydroxylase (TH), tryptophan hydroxylase (TPH)), receptors (5-HTRs, α_{1D} R), transporters (SERT, NET), are expressed at the stem cell stage, but are dormant, and their levels do not vary along both differentiation programs. This indicates that post-transcriptional mechanisms are at work when 1C11 cells convert into either serotonergic or noradrenergic cells.

Concerning the SERT mRNA, variations of the poly(A) tail length at the 3'-end occur from day 1 of the serotonergic program. It increases and reaches its maximal length on day 3 corresponding to the beginning of the SERT translation [33]. The length of the poly(A) tail then decreases at day 4. As blocking the 5-HT_{2B}R by ritanserin reduces the intensity of serotonergic functions at day 4, whether 5-HT_{2B}Rs control the polyadenylation step of the SERT mRNA is an attractive hypothesis that deserves further investigations.

In 2010 we provided evidence, using fluoxetine, that a microRNA (miR-16) governs the onset of serotonergic functions. We identified miR-16 as a negative regulator of SERT translation through miR-16 interaction with the 3' untranslated region (3'UTR) of SERT mRNA [35]. Higher levels of miR-16 were detected in 1C11^{NE} cells as compared to 1C11^{5-HT} cells. Such a disequilibrium in miR-16 levels had also been found *in vivo* in the noradrenergic neurons of the locus coeruleus *vs.* the serotonergic neurons of the raphe nuclei. In noradrenergic neurons, this high level of miR-16 prevents SERT expression as neutralization of miR-16 in 1C11^{NE} cells unlocks SERT translation and renders noradrenergic neurons competent to bind SSRI antidepressants. More surprisingly, miR-16 reduction in 1C11^{NE} cells also unlocks other serotonergic functions. In addition to SERT, the noradrenergic cells become capable to synthesize and catabolize 5-HT and express 5-HT_{2B}Rs. As no binding site for miR-16 was found in the 3'UTR of 5-HT_{2B}R mRNA, the molecular mechanisms by which miR-16 controls the onset of these serotonergic-specific functions are still unknown. *In vivo*, the injection of fluoxetine in mouse raphe provokes a rise in miR-16 levels in serotonergic neurons, thereby leading to a reduction of SERT expression [35], as observed in Prozac[®]-treated patients [36]. This SSRI antidepressant also induces the release of the neurotrophic factor S100 β by serotonergic neurons, which mediates the action of fluoxetine on noradrenergic neurons [35]. S100 β downregulates miR-16 in the locus coeruleus, which in turn, unlocks the expression of SERT as well as those of TPH and 5-HT_{2B}R. Noradrenergic neurons of the locus coeruleus

thus become a new source of 5-HT in the brain. The key role of miR-16 in the antidepressant action of fluoxetine was reinforced by studies performed in rodent models of depression. Increase of miR-16 in raphe or decrease of miR-16 in locus coeruleus improves depressive states similarly as fluoxetine [35, 37, 38]. Interestingly, 5-HT_{2B}R knockout mice display depressive-like behaviors and refractoriness to SSRI treatments [18, 39], indicating that 5-HT_{2B}R signaling contributes to antidepressant effects. Whether 5-HT_{2B}Rs implemented in noradrenergic neurons after fluoxetine/Prozac[®] treatment pilot the onset of 5-HT synthesis in the locus coeruleus at the origin of a new central source of 5-HT remains to be investigated.

2.3 Identification of 5-HT_{2B}R couplings along the 1C11 serotonergic differentiation

With the help of the 1C11 cell line, we evidenced that at day 2 of the serotonergic program, the 5-HT_{2B}R recruits the phospholipase A2 (PLA2)-arachidonic acid (AA) pathway [40] and the phospholipase C (PLC)- inositol-1,4,5-trisphosphate (IP3) pathway through the Gq proteins [27]. This latter coupling is lost at day 4 when 1C11^{5-HT} cells reach their terminal stage of differentiation [40]. The 5-HT_{2B}R also directly mobilizes the constitutive NO (cNOS) and the inducible NO (iNOS) synthases via the PDZ motif of its C-terminal extremity [41]. Interestingly, a special feature of the 5-HT_{2B}R among others 5-HT₂ receptors is to exhibit an intrinsic activity towards the couplings to PLC-IP3 [27], nitric oxide (NO) [41], PLA2-AA [40] and p21ras [42], which gives the receptor a major autocrine role during the serotonergic differentiation program. As described previously, the 5-HT_{1B/1D}R via G_i proteins is negatively coupled to adenylyl cyclase. At day 4, the 5-HT_{2A}R is coupled to the PLC-IP3 and PLA2-AA cascades [29, 40]. We further demonstrated the occurrence of crosstalks between the three 5-HT receptor subtypes in 1C11^{5-HT} cells. The 5-HT_{2B}R exerts an inhibitory effect on the 5-HT_{1B/1D}-mediated G_i coupling, which is relieved upon concomitant stimulation of 5-HT_{2A}R [40].

Through their couplings, all the three 5-HT autoreceptors play an essential role in modulating the intensities of 5-HT associated functions *i.e.* 5-HT synthesis, storage, catabolism and transport.

2.4 The 5-HT_{2B}R acts as a 5-HT biosensor that adjusts 5-HT levels by controlling SERT functionality

In vivo, plasmatic 5-HT concentration is tightly controlled to be maintained below 2 nM. When 1C11 cells are differentiated along the serotonergic pathway in a media containing high level of 5-HT (0.5-1 μ M), all 5-HT functions *i.e.* synthesis, storage and transport are reduced compared to 1C11 cells differentiated in presence of low level of 5-HT (< 1 nM) [28]. This reveals a negative feedback loop exerted by 5-HT that tones down the intensity of neurotransmitter-associated functions. This negative feedback loop notably relies on the 5-HT_{2B}R that was shown to behave as a biosensor of external 5-HT concentration and to control 5-HT levels by acting on 5-HT transport (SERT) and 5-HT catabolism.

As previously mentioned, the 5-HT transporter (SERT) is responsible for the reuptake of 5-HT across the plasma membrane and ensures a fine-tuned control of extracellular 5-HT concentrations. Deregulation of this precise control has been associated to diverse psychiatric diseases such as depression, anxiety and obsessive-compulsive disorders, characterized notably by reduced extracellular 5-HT levels in the brain. SSRI antidepressants used in clinics to combat depressive states block SERT function leading to a rise of central concentration of 5-HT. On the opposite, the serotonin syndrome relates to an excess of 5-HT in the central nervous system that may occur after therapeutic drug use causing adverse effects such as tremor, diarrhea, delirium, neuromuscular rigidity [43]. Exploiting the properties of the 1C11 neuronal stem cell, we firstly evidenced that the 5-HT_{2B}R governs SERT functionality (*i.e.* 5-HT transport and antidepressant recognition) through phosphorylation-type post-translational

modifications (Fig. 2) [33]. At low 5-HT concentration (1-2 nM, as in *in vivo* conditions), the intrinsic 5-HT_{2B}R coupling to NO production governs SERT phosphorylation to basal level in 1C11^{5-HT} neuronal cells. All the SERT molecules are functional allowing a maximal 5-HT uptake and the binding of SSRI antidepressants. In excess of 5-HT, the agonist-dependent 5-HT_{2B}R-IP3/PKC coupling promotes additional phosphorylation of SERT, which reduces 5-HT transport efficacy. This critical role of the 5-HT_{2B}R on SERT function was also evidenced in primary serotonergic neurons derived from the raphe. Another consequence of 5-HT_{2B}R-mediated hyperphosphorylation of neosynthesized SERT is the impairment of antidepressant recognition in 1C11^{5-HT} cells. In addition to SERT, the 5-HT_{2B}R via PKC signaling phosphorylates the energy source of the SERT, the Na/K ATPase electrogenic pump. This leads to a decrease of the Na/K ATPase pump activity that also contributes to the reduction of 5-HT transport. In serotonergic neurons, the autoreceptor 5-HT_{2B}R is thus a crucial regulator of 5-HT transport and sensitivity of SERT to antidepressants. As the Na/K ATPase may influence other transporters, it is likely that the 5-HT_{2B}R could contribute to the regulation of other yet to be identified neuronal and non-neuronal functions.

The 1C11 cell system has also been instrumental to identify a functional coupling between the 5-HT_{2B}R and the NADPH oxidase/TACE/TNF α that contributes to the control of 5-HT catabolism [44, 45]. By contrast to its PLC/IP3 and PLA2/AA couplings, the capacity of 5-HT_{2B}R to recruit NADPH oxidase and produce reactive oxygen species (ROS) is restricted to fully differentiated serotonergic 1C11^{5-HT} neuronal cells, suggesting that this bioaminergic receptor contributes to the maintenance of the redox equilibrium in mature serotonergic neurons only. This neurospecificity may relate to regulatory processes controlling partner assembly in lipid rafts during neuronal differentiation. Upon agonist stimulation of the 5-HT_{2B}R, NADPH oxidase-dependent ROS act as second message signals and govern the activation of the metalloproteinase TACE (TNF α converting enzyme, ADAM17). Activated

TACE ensures the shedding of soluble TNF α , which, in turn, increases the degradation of 5-HT into 5-HIAA in 1C11^{5-HT} cells [44, 45]. The link between the 5-HT_{2B}R and the NADPH oxidase-TACE-TNF α pathway indicates that the 5-HT_{2B}R autoreceptors may play an important role in the fine-tuning of 5-HT-associated metabolism.

2.5 5-HT_{2B}R signaling is modulated by the cellular prion protein in serotonergic 1C11 cells

As 5-HT_{2B}Rs exert a critical role as 5-HT biosensor and regulator of the intensities of 5-HT-associated functions, any modulators of 5-HT_{2B}R signaling will influence external 5-HT levels. To date, β -arrestin 2 was shown to be a negative regulator of 5-HT_{2B}R-mediated signaling by promoting its agonist-dependent internalization in LMTK-transfected cells [46]. In cardiac fibroblasts, it has been reported that the 5-HT_{2B}Rs interacts with angiotensin receptor AT1 to form heterodimeric complexes that impact cytokine release [47]. In 2005, we evidenced functional interactions between the cellular prion protein (PrP^C) and 5-HT receptors (Fig. 3) [48]. PrP^C, the normal isoform of the pathogenic scrapie prion protein (PrP^{Sc}) at the route of prion diseases, is a ubiquitous glycosylphosphatidylinositol (GPI)-anchored glycoprotein that exerts at the plasma membrane a role of receptor/co-receptor and is involved in signaling events [49]. Over the last decades, several signaling targets controlled by PrP^C have been identified such as PI3 kinase, PKC, NADPH oxidase, TACE, MAP kinases ERK1/2 [50]. A feature of the PrP^C-dependent neuronal response lies with the successful implementation of the signaling platform PrP^C/caveolin/Fyn kinase in cholesterol- and glycosphingolipid-rich lipid rafts of neurites [49]. Of note, bioaminergic receptors are also associated with such membrane microdomains. We demonstrated that in 1C11^{5-HT} cells, the antibody-mediated-stimulation of PrP^C combined with the agonist-dependent stimulation of 5-HT receptors abolishes the 5-HT_{2A}R-PLC coupling, reduces the intensity of the 5-

HT_{1B/1D}R negative coupling to adenylate cyclase, improves the efficacy of the 5-HT_{2B}R-PLA2 coupling and slightly restores the agonist-dependent 5-HT_{2B}R/PLC/IP3 coupling that is normally lost at day 4 of the serotonergic differentiation (Fig. 3) [48]. In these conditions, the 5-HT_{2A}R is no longer able to counterbalance the negative regulation of 5-HT_{2B}R on 5-HT_{1B/1D}R functions. The activation of PrP^C only disturbs receptor-couplings mobilizing G proteins. The 5-HT_{2B}R-NO coupling through the PDZ motif is not affected by PrP^C stimulation. Further, the impact of PrP^C on 5-HTR functionality is restricted to fully differentiated neurons, as at day 2 of the serotonergic program of 1C11 cells, PrP^C activation does not modify 5-HT_{2B}R and 5-HT_{1B/1D}R couplings. Therefore, it is unlikely that the modulation of cross-talks results from a direct interaction of PrP^C with 5-HTRs. Supporting this idea is the absence of any effect of PrP^C stimulation on the binding affinity of agonists and antagonists for 5-HT receptors. Rather, PrP^C action on 5-HTR signaling depends on PrP^C coupling to caveolin in 1C11^{5-HT} cells as the immunosequestration of caveolin abrogates the modulatory effect of PrP^C on 5-HTRs. By mobilizing caveolin, PrP^C could change the dynamic of interaction between signaling partners in rafts and modify the stoichiometry of G proteins recruited in response to 5-HTR activation. PrP^C thus emerges as a physiological modulator of serotonergic functions, possibly acting as a receptor activity-modifying protein (RAMP) that interferes with the 5-HT autoreceptors-coupled signaling pathways.

It is widely recognized that the interaction of PrP^C with pathogenic prions PrP^{Sc} and its conversion into PrP^{Sc} is at the origin of prion diseases [50-52]. For ten years, PrP^C is also known as a high affinity receptor for A β oligomers found in the Alzheimer's disease that relays, at least in part, A β toxicity [53-57]. More recently, PrP^C was shown to interact with the pathological α synuclein involved in Parkinson's disease [58]. The role of PrP^C in the modulation of 5-HT receptor-mediated signalings thus raises the question of whether the corruption of PrP^C by PrP^{Sc}, A β or α synuclein would be at the root of deregulation of 5-HT

receptor signaling pathways that could lead to a loss of homeostasis in serotonergic neurons and contribute to neurodegeneration. There are already evidences that prion infection alters 5-HT functions [59].

3 Role of the 5-HT_{2B} receptor in mineralized tissues

3.1 The 5-HT_{2B}R controls TNAP activity during osteogenic differentiation of the C1 mesodermic stem cells

The C1 tripotential mesoblastic cell line is endowed with the ability to recapitulate *in vitro* the spatio-temporal features of osteogenic, chondrogenic or adipogenic differentiations, under defined culture conditions [60]. The growth of C1 cells in three-dimensional nodules, mimicking the *in vivo* mesodermal condensation required for bone development and repair, is compulsory for cells to engage into osteogenic or chondrogenic programs. C1 aggregates, upon addition of β -glycerophosphate and ascorbate, start to produce an abundant extracellular matrix of type 1 collagen. The mineralization of this matrix initiates at day 7 by the deposition of hydroxyapatite crystals on collagen fibrils. At the end of the osteogenic differentiation (day 12), mature C1 osteocyte-like cells are embedded in a calcified matrix and stop to divide [61]. A functional 5-HT_{2B}R is induced at day 5 of the C1 osteogenic differentiation prior starting mineralization (Fig. 4) [62, 63]. From its implementation to the end stage of the osteogenic program, 5-HT_{2B}Rs are coupled to NOS/NO and PLA2/AA signaling pathways. Concerning AA metabolism, cyclooxygenase (COX) ensures the conversion of AA into prostaglandin PGE2 from day 5 to day 10. During the late phase of mineralization process, *i.e.* when osteoblasts are converted into osteocyte-like cells, COX activity is quenched by yet unidentified molecular mechanisms. From day 10 to day 12, AA are then metabolized by lipoxygenase leading to leukotriene (LTB4) synthesis. Both NO, PGE2 and LTB4 as intra- and/or inter-cellular second messengers, are well-known protagonists of bone homeostasis [64-

66]. They ensure either bone-forming and/or bone-resorbing effects. The major role of the 5-HT_{2B}R and its downstream signalings in matrix mineralization was evidenced by a 25-40% reduction of Ca²⁺ incorporation within the bone matrix using ritanserin, an agonist inverse of 5-HT₂Rs, or upon inhibition of NOS and COX. The involvement of the 5-HT_{2B}R in osteogenesis is also supported *in vivo* by the skeleton damages observed in 5-HT_{2B}R knock-out mice. Indeed, female 5-HT_{2B}R^{-/-} mice exhibit reduced bone mineral density with age, likely due to failure of osteoblast recruitment and/or proliferation [67].

We further demonstrated that 5-HT_{2B}Rs control bone mineralization by governing the activity of the GPI-anchored tissue-non-specific alkaline phosphatase (TNAP), a key player in bone formation (Fig. 4) [63]. As the 5-HT_{2B}R, TNAP is translated at day 5 of the C1 osteogenic program. Of note, both 5-HT_{2B}R and TNAP mRNAs are expressed as early as mesoblastic stem stage and their amounts remain unchanged throughout the 12 days of the differentiation. The signal events that switch on the translation of 5-HT_{2B}R and TNAP mRNAs in this specific timeframe are yet unknown. At day 5, TNAP is under an inactive form. This enzyme is activated from the start of mineral deposition on day 7 until the end-stage of the C1 osteogenic program by phosphatidylinositol-specific phospholipase C (PIPLC)-dependent post-translational mechanisms governed by the 5-HT_{2B}R/PLA2/eicosanoids signaling. In agreement, primary calvarial osteoblasts derived from 5-HT_{2B}R^{-/-} mice display defects in TNAP activity [63, 67]. Under physiological conditions, the 5-HT_{2B}R thus exerts a key role in mineralization processes. As PGE₂ and LTB₄ levels are altered in several bone diseases such as osteoporosis or rheumatoid arthritis [66], further investigations are needed to delineate whether deregulation of 5-HT_{2B}R signaling pathways would contribute to the development of these pathological situations.

3.2 Pulpal stem cell lines to reveal the critical role of serotonergic (5-HT_{2B,7R}) and dopaminergic (D_{1,3R}) autoreceptors in platelet-mediated tooth repair

During mouse embryogenesis, 5-HT exerts a critical role in craniofacial and tooth development, notably through the regulation of neural crest cell proliferation and migration from rhombomeres 1 and 2 [68]. An implication of the 5-HT_{2B}R on dental tissues was considered because of its expression in day-9 mouse embryos in the neuroepithelium and the mesenchyme of the first branchial arch which is at the origin of craniofacial bones and tooth buds [12]. Of note, 5-HT_{2B}R^{-/-} mice display structural alterations of teeth characterized by enhanced enamel porosities, thinner crystallites and disorganized rod structures [69, 70].

Fifteen years ago, clonal pulpal stem cell lines, such as the A4 and H8 cell lines, were derived from the first molar tooth germs of day 18 mouse embryos [71]. At the precursor state, A4 and H8 cells expressed odontogenic markers such as dentin matrix protein (DMP1), dentin sialoprotein (DSP), type 1 collagen and the LIM-domain homeobox transcription factors Lhx6 and Lhx7 which are present in the first branchial arch. *In vitro*, A4 cells are able to engage into odonto/osteogenic, chondrogenic and adipogenic differentiation and thus correspond to a multipotent mesoblastic stem cell. H8 cells whose potential of differentiation is limited to the odontogenic program, behaves as a monopotent precursor [72]. *In vivo*, both A4 and H8 pulpal stem cells favor an efficient tooth repair after implantation in injured mouse incisor or rat molar [72, 73].

Remarkably, although A4 and H8 cells are progenitors at different stages of commitment along the odontogenic lineage, both pulpal stem cells exhibit a dual serotonergic/dopaminergic phenotype (Fig. 5) [74]. A4 and H8 cells have the capacity to synthesize, catabolize, store and transport 5-HT and dopamine (DA). Further, they exhibit the same pattern of serotonergic and dopaminergic receptors at the plasma membrane. A4 and H8 cells expressed three serotonergic receptors, the 5-HT_{2B}R as well as the 5-HT_{1D}R and 5-

HT₇R, and two dopaminergic receptors, the D₁ and D₃ subtypes. In pulpal stem cells, the 5-HT_{2B}R recruits the PLC/IP3, PLA2/AA as well as the NOS/NO pathways. The four other bioaminergic receptors (5-HT_{1D,7}Rs, D_{1,3}Rs) are functionally coupled to adenylate cyclase/AMPC signaling, two positively (5-HT₇R, D₁R) and two negatively (5-HT_{1D}R, D₃R). Such a specific repertoire of 5-HT/DA receptors renders odontogenic stem cells capable to respond to 5-HT and DA in an autocrine and/or paracrine manner. How 5-HT/DA receptors signaling pathways interplay physiologically to ensure pulpal stem cell homeostasis and/or the balance between proliferation/differentiation remains to be investigated.

In tooth, the dental pulp is a highly vascularized tissue [75]. Of note, peripheral systemic 5-HT and DA are predominately stored in dense granules of blood platelets [76]. We demonstrated *in vivo* that in pathological situations, such as tooth injury, expression of the special 5-HT/DA receptor register allows odontogenic stem cells to be mobilized by circulating 5-HT and DA released by lesion-activated platelets for tooth repair (Fig. 5) [74]. In wild-type rats, a natural reparative dentin is formed at the exposure site 1 month after pulp lesion of the first maxillary molar. In the opposite, in Fawn-hooded and reserpine-treated rats, two rat models exhibiting a deficit of bioamine storage in platelets, reparative dentin formation is impaired. Thus, upon tooth injury, platelet-released 5-HT/DA represent essential "damage" signals for the recruitment of pulpal stem cells expressing 5-HT/DA receptors for tooth repair. The role of 5-HT_{2B,7}Rs and D_{1,3}Rs in dentin repair is further supported by the impairment of tooth reparative processes observed in wild-type rats after addition of selective antagonists for each receptor in the damaged molar pulp. Many questions remain related to the signaling pathways and downstream targets controlled by 5-HT_{2B,7}Rs and D_{1,3}Rs to orchestrate the mobilization/proliferation/differentiation of odontogenic stem cells for dental repair.

4 Conclusion

This review focuses on clonal neuronal and mesoblastic stem cell lines with homogeneous differentiation properties allowing the identification of several signaling pathways and key effectors governed by the 5-HT_{2B}R for the fine-tuned coordination of cell homeostasis and differentiation. These lineage progenitors used as test tubes also led to build pathophysiological scenarii implicating the 5-HT_{2B}R and provided some clues as to the events involved in disease-associated states such as depression, ectopic mineralization or tooth repair. Indeed, the 1C11 neuronal stem cell line was notably useful to reveal the key role of miR-16 as a relay of fluoxetine action both on raphe serotonergic neurons and noradrenergic neurons of the locus coeruleus. Fluoxetine, through miR-16-dependent unlocking of serotonergic functions (5-HT synthesis, 5-HT_{2B}R) in the locus coeruleus can activate a new source of 5-HT in the brain, a breakthrough in understanding the mode of action of SSRIs. In the dental field, although many challenges remain, highlighting the crucial role of the dialogue between pulp injury-dependent release of 5-HT/DA from platelets and 5-HTRs/DARs-expressing odontogenic stem cells for tooth repair could pave the road for novel therapeutic strategies.

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Conflicts of interest

The authors declare no conflict of interest.

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Figures Legends

Fig. 1 The inducible 1C11 neuronal stem cells have the capacity to differentiate into serotonergic (1C11^{5-HT}) or noradrenergic (1C11^{NE}) neuronal cells in a mutually exclusive manner.

Fig. 2 By sensing external 5-HT levels the 5-HT_{2B}R controls SERT functionality in 1C11^{5-HT} neuronal cells through the phosphorylation of SERT and of its energy source, the Na/K ATPase.

Fig. 3 PrP^C modulates 5-HT_{2A,2B,1B/D} receptors signaling couplings in 1C11^{5-HT} neuronal cells. In 1C11 serotonergic cells at day 4, co-stimulation of PrP^C with antibodies and 5-HTR with specific agonists impacts on G-dependent 5-HTR-couplings (see text for details).

Fig. 4 The coupling of 5-HT_{2B}R to the PLA2/eicosanoids/PIPLC pathway controls TNAP activity during osteogenic differentiation of the C1 mesodermic stem cells.

Fig. 5 5-HT and DA co-released by activated platelets upon tooth injury are "damage" signals that mobilize pulpal stem cells expressing 5-HT_{2B,1D,7/D_{1,3}} receptors for tooth repair.









