CROSSTALK BETWEEN THE OSTEOGENIC AND NEUROGENIC STEM CELL NICHES: HOW FAR ARE THEY FROM EACH OTHER?

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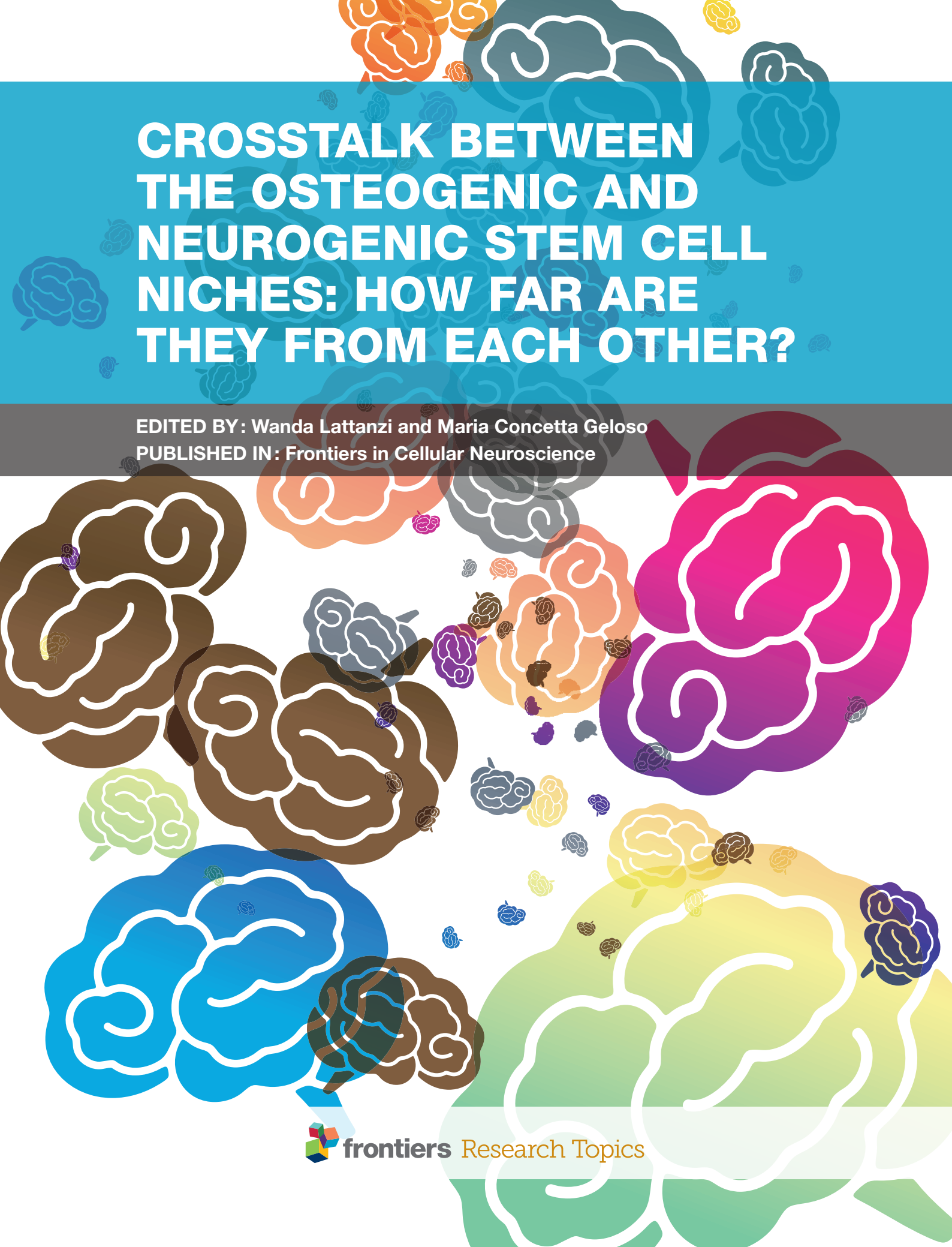

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CROSSTALK BETWEEN THE OSTEOGENIC AND NEUROGENIC STEM CELL NICHES: HOW FAR ARE THEY FROM EACH OTHER?

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Somatic stem cells reside in definite compartments, known as "niches", within developed organs and tissues, being able to renew themselves, differentiate and ensure tissue maintenance and repair. In contrast with the original dogmatic distinction between renewing and non-renewing tissues, somatic stem cells have been found in almost every human organ, including brain and heart.

The adult bone marrow, in particular, houses a complex multifunctional niche comprising hemopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), that intensely interact. HSCs represent the common precursors of all mature blood cells. MSCs are instead able to differentiate along multiple mesodermal lineages and are believed to represent the key somatic stem cell within the skeletogenic niche, being conceptually able to produce any tissue included within a mature skeletal segment (bone, cartilage, blood vessels, adipose tissue, and supporting connective stroma). Despite this high plasticity, the claim that MSCs could be capable of transdifferentiation along non-mesodermal lineages, including neurons, has been strongly argued. Adult osteogenic and neurogenic niches display wide differences: embryo origin, microenvironment, progenitors' lifespan, lineages of supporting cells. Although similar pathways may be involved, it is hard to believe that the osteogenic and neurogenic lineages can share functional features.

The outbreaking research achievements in the field of regenerative medicine, along with the pressing need for effective innovative tools for the treatment of neurodegeneration and neurologic disorders, have been forcing experimental clinical applications, which, despite their scientific weakness, have recently stimulated the public opinion.

Based on this contemporary background, this Research Topic wish to provide an in-depth revision of the state of the art on relevant scientific milestones addressing the differences and possible interconnections and overlaps, between the osteogenic and the neurogenic niches. Dissertations on both basic research and clinical aspects, along with ethical and regulatory issues on the use of somatic stem cells for *in vivo* transplantation, have been covered.

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EDITORIAL
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Editorial: Crosstalk between the Osteogenic and Neurogenic Stem Cell Niches: How Far are They from Each Other?

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Keywords: stem cell niche, osteogenic niche, neural stem cells (NSCs), mesenchymal stem cells (MSCs), regeneration

The Editorial on the Research Topic

Crosstalk between the osteogenic and neurogenic stem cell niches: how far are they from each other?

Despite the intense research on adult neural stem cell biology suggested possible translational outcomes in regenerative medicine for neurodegenerative diseases, neuroregeneration is unlikely to occur in adult brain, due to intrinsic features that characterize the neural stem cell niche.

Mesenchymal stem cells (MSCs), osteogenic stem cells residing in the bone marrow stroma (also named bone marrow stromal cells), have been long considered highly plastic multipotent precursors, able to commit toward diversified lineages, including non-mesodermal ones. Their *in vitro* plasticity and ease of processing prompted their wide, sometimes untimely, exploitation in diversified regenerative medicine applications (Park et al., 2012; Bianco et al., 2013). They have been tested also for their putative, yet widely debated, neuroregenerative potential. This controversial issue stimulated this Research Topic, which aims to delve into relevant scientific milestones addressing the differences, possible interconnections, and overlaps between the osteogenic and the neurogenic niches' biology.

The debated neuronal transdifferentiation potential of MSCs recently led to their inappropriate exploitation for the treatment of neurodegenerative disorders. The regulatory and ethical issues regarding this topic have been discussed in the Opinion paper by Solarino et al., delving into a recent Italian case of medical malpractice, which triggered significant international dispute (Abbott, 2013; Blasimme and Rial-Sebbag, 2013). Indeed, a better clarification of the specific features displayed by the osteogenic and the neurogenic stem cell niches is needed, as discussed by Lattanzi et al. This mini-review provides a pairwise comparison of the two niches within their *in vivo* environments, highlighting functionally relevant similarities and differences that should be considered to achieve a more rational clinical translation.

The contribution by Salgado et al. provides an exhaustive description of osteogenic and neural stem cells' features, focusing on their possible interaction within the brain environment. In particular, the MSCs' secretome is known to exert autocrine and paracrine effects that may be relevant for potential therapeutic exploitations, also in the central nervous system (Ribeiro et al., 2011; Drago et al., 2013; Kim et al., 2013; Sart et al., 2014; Wright et al., 2014).

The role of neural crest stem cells (NCSCs) in regulating the bone marrow niche is provided in the review by Coste et al. NCSCs are capable of epithelial-to-mesenchymal transition, and ultimately give rise to both neural precursors and nestin-positive MSCs, actively involved in the

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homeostatic regulation of the hematopoietic stem cell niche (Achilleos and Trainor, 2012; Mayor and Theveneau, 2013).

A significant overlap between the two niches relies on the molecular (Wnt, NOTCH, FGF, TGF-BMP, SHH signaling pathways) and secretome (BDNF, NGF, VEGF, PDGF) profiles, along with the intimate relationship with vessels, being a common structural feature observed in adult stem cell niches.

Diverse phylogenetically old signaling pathways, including nucleotides and neuropeptides, are shared between the osteogenic and the neurogenic niches, exerting trophic, and immunomodulatory functions. Cavaliere et al. exhaustively discussed the often opposing roles played by purinergic ligands. These establish a common paracrine pathway that modulates MSCs' and NSCs' activity, in both physiological and pathological conditions. They appear to be involved in the crosstalk between the two niches, by modulating the immune response, which triggers stem cell recruitment after stressful insults (Cavaliere et al.).

Among neuropeptides, the direct effects of neuropeptide Y (NPY), mediator for signaling in both neurogenic and osteogenic niches, has been reviewed by Geloso et al., with special attention to its effects on neurogenic niche. Data indicating a direct pro-neurogenic effect of NPY on NSCs, as well as the concomitant modulatory action on astrocytes, microglia, and endothelium activities within the niche have been discussed. Interestingly, a possible crosstalk between released nucleotides and NPY related pathways emerges (Jia and Hegg, 2012), suggesting that they could also represent a point of intersection between shared ancient molecular pathways.

Neurotransmitters released by the sympathetic nervous system, interestingly including NPY, as recently reviewed by Park et al. (2015), are known to be also involved in the regulation of hematopoietic stem cell (HSC) functions, mainly acting on endothelial cells and nestin-positive MSCs, which retain HSCs. In this regard, the relevance of catecholaminergic modulation of hematopoiesis has been extensively reviewed by Cosentino and coworkers (Cosentino et al.), highlighting their established role in the complex network of neural and neuroendocrine agents that regulate stem cell biology (Cosentino et al.).

Within the wide range of external stimuli acting on the epigenetic control of adult tissue stem cell niches, the effects of extremely-low frequency electromagnetic field (ELFEF) stimulation is emerging as a tool to modulate neurogenic and osteogenic processes, as discussed by Leone et al. They highlighted the possible shared pathways induced by ELFEFS on both niches, including Wnt/beta-catenin signaling and the activation of p300 or other histone acetyltransferases by Runx2 (Leone et al.).

The interdependence of brain and skull during development seems to rely also on the role of interposed meninges (Richtsmeier and Flaherty, 2013). Within this intriguing topic, Bifari et al. provided findings showing the distribution of neural precursor markers in rat meninges during development up to adulthood, related to the newly identified niche function of meninges (Decimo et al., 2011).

Finally, an interesting evolutionary perspective on the relation between osteogenesis and neurogenesis is provided in the opinion paper by Boeckx and Benítez-Burraco, who approached this topic from a different "biolinguistic" standpoint. The Authors postulated that critical genes active in the osteogenic niche (including homeogenes, e.g. DLXs, morphogens, e.g. BMPs, and the master regulatory RUNX2 gene), hence giving rise to skull globularity in anatomically modern humans, also have important consequences in brain development and plasticity, ultimately leading to our distinctive mode of cognition (Boeckx and Benítez-Burraco).

Taken together, the papers included in this research topic seem to suggest an emerging cross-domain scenario in which significant molecular signaling and biological features are shared between osteogenic and neurogenic stem cells niches. The two niches appear to be interconnected in evolution, during development, and further beyond. Nonetheless, relevant differences in the relative stem cell niche dynamics should not be neglected, in order to appropriately design potential cross-lineage tissue regenerative strategies.

AUTHOR CONTRIBUTIONS

Both Authors contributed equally in conceiving, drafting, revising, and finalizing the present manuscript.

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OPINION
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Stem cell therapy: medico-legal perspectives in Italy

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Keywords: mesenchymal stem cells, compassionate treatment, medical liability, legal dispute, stamina

Ethical and juridical issues have recently been raised in Italy regarding experimental stem cell therapy (Stamina), which was authorized but then stopped after it was administered to a wide range of patients as a compassionate treatment for neurodegenerative diseases (Carrozzi et al., 2012; Finkel, 2012; Mercuri and Bertini, 2012; Abbott, 2013; Cattaneo and D'Ambrosio-Lettieri, 2015).

Research in the field of somatic stem cells isolated from adult organs has been developed over the last few decades as a powerful tool in regenerative medicine. In particular, most experimental tissue regenerative applications are based on the use of mesenchymal stem cells (MSCs) to regenerate damaged tissues (Biffi et al., 2013). Some have claimed that MSCs could be capable of neuronal transdifferentiation, though this feature is poorly substantiated and widely contested (Brundin et al., 2010; Dyson and Barker, 2011; Lattanzi et al., 2011; Bianco et al., 2013; Urbán and Guillemot, 2014; van Velthoven et al., 2014).

Despite the fact that numerous ongoing studies and clinical trials have exploited such stem cells in the treatment of bone and soft tissue defects, no studies have investigated their possible application in the field of degenerative diseases affecting non-mesodermal organs. Hence, yielding promising results could produce higher expectations in poor prognosis patients and in their caregivers (Notarangelo, 2013; Campana et al., 2014; Reddington et al., 2014).

The complicated sequence of events of the so-called "Stamina" method has garnered keen public support, but equally, scientists' opposition. This has generated a long and complex investigation by the Public Prosecutor's office in Turin regarding accusations of criminal conspiracy aimed at fraud, unlawful medical practice, violation of privacy norms and many other crimes.

No details of these innovative protocols have been provided by the promoters of this method, who generically claimed that they were able to differentiate bone marrow MSCs into nerve cells for the treatment of neurological, genetic, and autoimmune diseases. The principles of these studies seem to derive from two Russian and Ukranian papers (Schegel'skaya et al., 2003; Yavorskaya et al., 2006). Only very recently, the results obtained in three patients have been described, although the description of the experimental protocol is still inadequate (Villanova and Bach, 2015).

However, this story finally caused the Public Health System to be involved in a legal dispute, as the method was claimed to represent a compassionate treatment, for which unlimited access should be granted. A compassionate therapy is administered when there is no alternative to the experimental therapy—in the broadest sense of the term, with the relevant variables—even in order to grant the patient and their relatives a dignified co-existence with a pathological condition which would otherwise be progressive, irreversible and lethal.

Indeed the protection of the right to health is attributed to the legislator even in deciding the financial allocation of taxpayers' funds so this right remains dependent on the choice of instruments, timing and implementation methods as foreseen by the law and by the administrative authorities. As a consequence, the access to a new therapy, even administered with a compassionate aim, as a matter of principle cannot be deemed individually unlimited, because it is regulated by healthcare norms that define the prerequisites of scientific validity and the "ethicalness" of the new therapy, including stem cells.

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A solid and efficient regulatory framework is required in Europe as the milestone for developing cell-based therapies (Blasimme and Rial-Sebbag, 2013). This is particularly true for the compassionate therapies in which the European Medicines Agency (EMA)—in the *Guidelines on Compassionate Use of Medicinal Products* [pursuant to article 83 of Regulation (Ec) no 726/2004]—states that it is only possible to collect data on safety *during compassionate programmes* but *such programmes cannot replace clinical trials* that *provide essential information relative to the benefit/risk balance of a medicinal product.*

In the light of this, it is still not clear on what scientific bases the unknown Stamina Method was authorized by the Ethic Committee at the Spedali Civili in Brescia, despite the fact that the quite alarming results of the inspection carried out by the Italian Medicine Agency—AIFA (a body entitled to grant access to drugs and to supervise the correct and safe use of drugs)—prohibited any immediate and effective *sample taking, transport, handling, cultures, stocking, and administering of human cells at the "Spedali Civili" hospital in Brescia* promoted by the Stamina Foundation.

The Regional Administrative Court (TAR) of Brescia (9th of September 2012) confirmed the "lack of scientific evidence," the omitted transmission of the data to the Italian National Institute of Health and the absence of valid opinions of the Ethics Committee for each of the treated patients.

However, in the *uneasy pondering of the interests at stake*—on the one hand patients' interest in continuing the so-called compassionate therapy inhibited by AIFA, on the other the power of this agency to regulate the experimentation of new drugs—the TAR considered decisive the *unlikelihood of getting to know the production method and the therapeutic use of mesenchymal cells used by "Stamina" which, moreover, is not acknowledged to be valid by the national and international scientific community*.

Therefore, the only way of continuing the therapies was through the implementation of adequate judiciary measures. This led to a proliferation of urgent appeals to the Labor Law Judge, who has jurisdiction over matters of mandatory medical assistance; but these appeals were aimed at obtaining from the hospital in a compulsory way the administration of stem cells without any proven therapeutic efficacy, thus causing the irreparable violation of the primary and constitutionally guaranteed right to health and life of the patients affected by terminal pathologies and/or negative prognoses.

The complex legal framework has not even been solved by the approval of Law Decree n. 24/2013, converted with modifications by Law n. 57/2013. This law—because of the *deep anguish of the patients, who hope to obtain from the Stamina therapy those benefits in terms of health which, because of the serious [nature of the] diseases under discussion, cannot be provided by the use of already approved drugs or at least already experimented drugs* and because of the absence of *serious side effects*—allowed only for the continuation, under the National Health Service conditions, of the stem cell therapies.

In opposition to the judges' authorization, imposing the injections, two independent scientific committees were appointed by the Minister. They expressed their negative opinion because *the Stamina method for the preparation of MSCs is not adequate. The MSCs produced with the Stamina method do not satisfy the requirements for the definition of these cells as therapeutic agents. The proposed Stamina protocols do not satisfy the basic requirements for any clinical experimentation because the Stamina method and control do not possess the scientific requisites necessary to carry out a clinical trial, including the evaluation of the safety and effectiveness [and therefore] the conditions to begin the experimentation with the so-called Stamina method, in particular the patients' safety, do not exist.* The Health Ministry, consequently, with a note dated 4 November 2014, has acknowledged that *the experimentation [. . .] cannot be continued further.*

The role of the Courts in ordering the physicians to provide the experimental treatment, especially to a vulnerable population, was largely criticized by the scientific community (Finkel, 2012). In comparison with the proclaimed results of the Stamina method, other scientists began a compassionate therapy, administering intrathecal MSCs in children with Type I spinal muscular atrophy (SMA). Because of the lack of efficacy the Hospital, in accordance with the local Ethical Committee, stopped the recruitment of patients for this kind of therapy (Carrozzi et al., 2012). The scientists highlighted the risk that the *combination of newspaper hype and parental hope, with the support of the Courts that are sympathetic to families with children with severe disorders,* may produce a lack of scientific evidence in conflict with the common rules of clinical investigation.

From this perspective, one can notice a significant similarity with what often happens in cases regarding the side effects of vaccines, which have generated several different rulings on the unidentifiable nature of the damage most likely to be seen in a causal correlation with the administration of the vaccine.

In both hypotheses, what "recedes" in the face of a health or a life threat are not only the legislative and administrative powers to allocate—limited—resources for health matters, imposing certain services and prohibiting others, but also the scientific validity of the treatments themselves, which constitutes the ineluctable rational requirement for the exercise of that power.

Each time science does not give univocal answers—which means almost always in medicine—the lack of access to compassionate treatments may lead to an irreparable violation of the right to health and to human dignity. This is true for the administration of whatever drug may have even a vague and controversial chance of success or even just palliative effects (in other words, imposing an indemnification in the case of pathologies whose correlation with vaccines may be possible but not demonstrable).

Therefore, the judge in these cases does not invent science: s/he simply disregards it.

This approach may be debatable and it has recently been disregarded by the Italian Constitutional Court (274/14), which recalled that the decisions regarding therapeutic choices, specifically addressing their adequacy, cannot arise from the politically discretional evaluations of the legislator, but must be founded *on the verification of the state of scientific knowledge and the experimental evidence acquired by institutions and bodies—usually national and supranational—in charge of doing so, considering the essential matter with which the technical-scientific bodies deal.*

Based on these preconditions, the judge affirmed that the clinical experimentation of a new drug does not normally allow charging in advance the public bodies with the duty to administer the drug either for the need to safeguard health or for the need to guarantee the correct allocation of funds available from the National Health Service.

As a consequence, the continuation of the therapies with the Stamina method establishes a waiver, due to its nature as an exception, which does not set up any irrational disparity in the treatment for those patients who ask for access to compassionate therapies which are no longer allowed because they lack an adequate technical-scientific support.

In the same way, the European Court of Human Rights (Durisotto v. Italy—application no. 62804/13) has ruled that the prohibition on access to the therapy, imposed by an Italian court in application of legislative decree no. 24/2013, did not violate any human right because it pursued the legitimate aim of protecting health, was proportionate to that aim and was neither arbitrary nor discriminatory.

If not even the judges can disregard science, certainly doctors cannot disregard it.

It is a fact that stem cell treatment is used in certain human conditions; however physicians who prescribe and administer the new treatment need to understand the basic principles of this study. In the Stamina method we firstly have to ask how bone marrow cells can be converted into nerve cells or can promote blood vessel growth.

So, which norm applies to this case, since we have a judiciary measure which, hypothesizing, conflicts with the obligations of *diagnostic therapeutic autonomy and responsibility* established by the latest edition (2014) of the Italian Code of Medical Ethics (art. 22 *the doctor whom one asks for services which are in conflict with their conscience or with their clinical convictions can deny his/her services unless this behavior is a serious or immediate threat to the health of the patient, and must provide citizens with all useful information and clarification*)?

The prosecutor's investigation revealed that the doctors who were injecting the product in the patients did not appear to be aware of the real nature of the biological material that was being administered. Should the doctors at the "Spedali Civili" in Brescia, who have declared to the special commissioner of the hospital their refusal to administer the Stamina imposed by judicial measures, be subject to penal sanctions for nonfeasance (art. 328 penal code: the person in charge of a public service who wrongfully denies the service of which they are in charge, and which, for judicial, public safety, public order or hygiene or health reasons must be performed without delay, is sanctioned with imprisonment for a period of 6 months to 2 years)?

The answer, in our opinion, must be negative. And this is because in these cases the refusal cannot be considered wrongful, but, on the contrary, is founded on a due justification in one's professional field as well as on the law and on the regulations of the appropriate body (AIFA).

The history of this new "sensational" treatment ended with the head of the project negotiating a plea bargain.

Concluding Remarks

In short, the improvement of stem cell experimental therapy needs rigid juridical rather than scientific boundaries. Scientists have a fundamental role in communicating the aims coupled with the limitations of their ongoing studies. This means that the usefulness of stem cells can be affirmed with caution, especially in the case of compassionate therapies, strictly following the guidelines imposed by the regulatory authority. The judges have the great responsibility to agree with the best scientific evidence without imposing their own "personal" interpretation of science simply to meet the social expectations of poor prognosis patients and of their caregivers. Moreover, they must punish the defendants who make false claims about a given therapy, playing on patients' vulnerabilities. Many of these sensational therapies hide economic interests that are "paid for" by the patients and the community as a whole. The politicians have the institutional function not to ride the wave of the moment but to guarantee the constitutional right of each patient to make their own healthcare decisions based upon solid scientific findings. Finally doctors may help patients to understand the meaning of compassionate therapy that can never be separate from scientific methodology and evidence.

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Osteogenic and Neurogenic Stem Cells in Their Own Place: Unraveling Differences and Similarities Between Niches

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Although therapeutic use of stem cells (SCs) is already available in some tissues (cornea, blood, and skin), in most organs we are far from reaching the translational goal of regenerative medicine. In the nervous system, due to intrinsic features which make it refractory to regeneration/repair, it is very hard to obtain functionally integrated regenerative outcomes, even starting from its own SCs (the neural stem cells; NSCs). Besides NSCs, mesenchymal stem cells (MSCs) have also been proposed for therapeutic purposes in neurological diseases. Yet, direct (regenerative) and indirect (bystander) effects are often confused, as are MSCs and bone marrow-derived (stromal, osteogenic) stem cells (BMSCs), whose plasticity is actually overestimated (i.e., trans-differentiation along non-mesodermal lineages, including neural fates). In order to better understand failure in the "regenerative" use of SCs for neurological disorders, it could be helpful to understand how NSCs and BMSCs have adapted to their respective organ niches. In this perspective, here the adult osteogenic and neurogenic niches are considered and compared within their *in vivo* environment.

Keywords: brain repair, neurodegenerative diseases, neural stem cells, mesenchymal stem cells, adult neurogenesis, osteogenesis

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INTRODUCTION

Stem cells (SCs) are considered "functional states" rather than "cell types" with a specific morphology and function, these being features more typical of mature cells (Morrison and Spradling, 2008). SCs act dynamically in tissue development, renewal, and regeneration, their activity and fate being regulated by molecular and cell-to-cell contact signals from the surrounding environment. Hence, somatic SCs in adult organs live within – and need – highly regulated, morpho-functionally defined microenvironments known as niches (Scadden, 2014). During development and growth, these niches remain "trapped" within tissue architectures throughout the body. As a result, different niches populate the organs and display variations of a common theme, sharing features which "adapt" to different functional demands. In spite of a vast amount of research, it remains largely unknown how diverse SCs and their niches function *in vivo* within different organs. By contrast, *in vitro* research on SC biology has been characterized by repeated breakthroughs, resulting in the perception that SCs can easily cure many diseases (Bianco et al., 2013a,b; Cattaneo and Bonfanti,

2014). At present, however, only selected populations of adult SCs are able to repair a limited number of skin, cornea, and blood pathologies, being of limited use in other contexts. Despite a lack of reliable evidence, statements in the media and even scientific papers have emphasized the use of "mesenchymal" stem cells (MSCs) such as those residing in the bone marrow (BM) stroma, as a source of trans-differentiating elements capable of colonizing different organs (including the brain) to replace lost cells. On these bases, MSCs have often been presented as elements which could overcome the strict rules regulating the SC niche/tissue relationships, even if most of their regenerative outcomes have not been confirmed by subsequent studies, since "MSCs commonly defined by *in vitro* functions have entered clinical application despite little definition of their function in residence" (Park et al., 2012). In addition, MSCs are usually considered as the osteogenic SCs residing in the BM stroma. Nonetheless, the term "mesenchymal" is now considered inappropriate as these adult SCs are biologically distinct from the embryo "mesenchyme"; accordingly, they are called bone marrow stromal cells instead (BMSCs; Bianco and Robey, 2015). Beyond semantics, the sometimes confusing terminology used to define these cells reflects the complexity of their biology and the cellular heterogeneity of their niche.

The misunderstandings become even more astonishing if such cells are employed to heal neurological diseases, since the central nervous system (CNS), although hosting neural stem cells (NSCs), remains refractory to repair/regeneration (Bonfanti, 2011; Peretto and Bonfanti, 2014). This review outlines the state-of-the-art regarding the inherent specificity of osteogenic and neurogenic niches through a detailed comparison of the microenvironment housing stromal (osteogenic) and NSCs, as well as their outcome in physiological and regenerative conditions.

SKELETAL STEM CELLS AND THEIR OSTEOGENIC NICHES

Although bone biology is apparently understood, an unambiguous setting for the osteogenic niche still represents a conundrum, hardly unraveled even after extensive revision of the relevant scientific literature. Bones, as complex organs, in mammalian vertebrates involve distinct specialized tissues: bone, cartilage, adipose tissue, blood vessels, all derived from multipotent BMSCs, along with BM and nerves. Bone, as a tissue, is a specialized connective containing osteoblasts, osteocytes, and osteoclasts, which cohabit and maintain a mineralized supporting matrix. After birth, bones still grow to achieve the final size of the skeleton, through either endochondral (bone replaces a cartilaginous bud in long bones) or membranous (connective membranes in the skull vault are directly converted into bone tissue) ossification. Even beyond completion of ossification, all bones are still extremely plastic and capable of adaptation to mechanical forces and chemical stimuli: they increase their sizes through cortical modeling (bone apposition on external surfaces) and modify their shape through remodeling (coupled bone apposition and resorption). These processes persist in adulthood, though modeling activity significantly decreases after peak bone mass is achieved, with a chronology that varies in different species, due to the variable lifespan and mechanics (Hall, 2014).

Osteogenic niches are found throughout the skeleton. Although no data are available on their actual number, it is reasonable to consider each single bone housing an organ-specific niche: over 200 quite large niches orchestrate tissue remodeling to maintain stable biomechanical conditions upon changing environmental stimuli (Long, 2011), with mature lineages being homeostatically renewed on a monthly basis (Long, 2011; Park et al., 2012).

Given this complexity, a univocal definition of the proper osteogenic niche is still pending. Converging evidence indicates BMSCs as the most upstream progenitors in the BM stroma. They were initially described as an adherent, fibroblastoid cell population with inherent osteogenic properties (Friedenstein et al., 1970). Although cells sharing features with BMSCs are found in other tissues (e.g., adipose tissue and skeletal muscle; Asakura et al., 2001; Zuk et al., 2001; Barba et al., 2013), BMSCs represent the best characterized cytotype (Park et al., 2012), able to self-renew and to generate multiple mesodermal lineages found within a skeletal segment (Bianco et al., 2013a,b). A specific subpopulation of BMSCs – namely, skeletal stem cells (SSCs) – is thought to represent the direct osteogenic SCs giving rise to the osteoblast/chondroblast lineage (Park et al., 2012; Chan et al., 2013; Bianco and Robey, 2015; see below). Conversely, the osteoclast lineage derives from hematopoietic stem cells (HSCs) through differentiation of monocyte/macrophage precursors. The osteogenic and hematopoietic niches are functionally related and mutually inter-dependent within the BM environment in trabecular bone: BMSCs and SSCs support and regulate HSCs homing *in vivo*; HSCs provide osteoclast precursors that combine with osteogenic lineage's cells to form bone structure (Morrison and Scadden, 2014).

Bone SCs are mostly found around the walls of BM sinusoidal vessels, close to pericytes, where they are thought to contribute to the formation of an "endosteal niche," on the vascularized endosteal lining of bones (Sacchetti et al., 2007). SSCs also reside in the inner layer of periosteum, which is also highly vascularized and innervated (De Bari et al., 2006; Roberts et al., 2015); herein, they drive endochondral ossification, contribute to bone modeling and remodeling in both long and flat bones (Kronenberg, 2003; Chan et al., 2009), and are crucial for bone regeneration during fracture healing (Colnot, 2009). Therefore, two apparently separate compartments can contribute to the adult osteogenic niche: an inner "endosteal domain" – with BMSCs and SSCs housing BM cavities and lining endosteal surfaces – and a "periosteal domain," being differently regulated and mediating different functions in bone homeostasis (Colnot, 2009). As periosteal vessels supply most of cortical bone vascularization, it is reasonable to consider blood vessels as the *trait d'union* between the two domains. Nonetheless, osteoprogenitors have been described also far from the typical perivascular location (Worthley et al., 2015).

The alternative ossification paths (endochondral and membranous), and corresponding embryo origins, suggest a regional segregation of niches (Schlecht et al., 2014). Most bones derive from the mesoderm through endochondral ossification, while skull bones originate from the neural crest (neuroectoderm), where highly migratory and plastic cells drive the membranous

(direct) ossification of the skull vault (calvarium), coordinate skull–brain development and growth (Richtsmeier and Flaherty, 2013), and persist after birth within the dense connective tissue forming skull sutures (Lana-Elola et al., 2007; Lattanzi et al., 2012). Therefore, calvarial bone's niches include endosteal and periosteal domains plus a "suture domain," which progressively disappear as sutures ossify (Schlecht et al., 2014; Zhao et al., 2015). Moreover, the dura mater meninx underlying skull bones houses multipotent cells as external niche contributors (Opperman et al., 1993; Merrill et al., 2006).

Comprehensive descriptions of the skeletogenic lineage arising from BMSCs allowed identifying subtle immuno-phenotype and commitment-related differences within the lineage sequence (Park et al., 2012; Chan et al., 2013). Nonetheless, the criteria for univocal classification of SSCs as distinct from BMSCs are still unstable and pending. Both cells are perivascular, share stemness surface markers (see **Table 1**), and display extensive *in vitro* multilineage potential (angiogenic, adipogenic, and osteogenic), in spite of an extremely limited plasticity *in vivo* (Park et al., 2012; Bianco et al., 2013a,b; Chan et al., 2013). BMSCs typically display long-term self-renewal capacity, though they self-renew at a much slower rate compared to blood and epithelia (Kassem and Bianco, 2015). They commit to osteogenic precursors by expressing additional lineage-specific marker genes, hence turning into proper SSCs (**Table 1**). SSCs are mitotic, self-renewing, "oligopotent" elements, giving rise to cell progenies of bone tissue (osteoblasts and chondrocytes; Bianco et al., 2013a,b; Chan et al., 2013). Subsequent osteoblast progenies are endowed with an intense cell renewal potential and undergo relatively rapid turnover (Park et al., 2012). The entire and complex BM niche is maintained through constant interactions with vasculature and stromal components that regulate self-renewal and differentiation of SCs and early progenitors (Méndez-Ferrer et al., 2010; Ding et al., 2012). This structural dualism within the BM niche enables direct paracrine signaling between HSC and SSC niches: bone progenitors and osteoblasts provide regulatory cues for HSC homing and maintenance of hematopoiesis (Arai and Suda, 2007).

In most mammals, bone activity changes during the entire lifespan of an individual, due to modification in the composition of the osteogenic niches. Cellularity decreases with age in all domains of the niche, as a consequence of reduced renewal of both BMSCs and early progenies (Muschler et al., 2001; Ochareon and Herring, 2011; Schlecht et al., 2014), BMSC plasticity being also impaired (Zhou et al., 2008; Choumerianou et al., 2010; Asumda and Chase, 2011).

NEURAL STEM CELLS AND THEIR NEUROGENIC NICHES

For a long time, the adult mammalian CNS has been considered unable to undergo cell renewal, since it is composed of "perennial" nerve cells (Colucci-D'Amato et al., 2006). Yet, populations of NSCs actually persist in some adult CNS regions (Reynolds and Weiss, 1992), producing undifferentiated neuronal and glial precursors (Gage, 2000; Kriegstein and Alvarez-Buylla, 2009; **Table 1**). Two brain areas generate new neurons that functionally integrate into neural circuits: the forebrain ventricular-subventricular zone (V-SVZ, or SVZ), the largest germinal region in the adult mammalian brain gives rise to olfactory bulb interneurons (Silva-Vargas et al., 2013); the subgranular zone (SGZ) of the hippocampus generates granule cells in the dentate gyrus (Aimone et al., 2014).

In the adult SVZ, NSCs are a population of special cells with certain astrocyte properties, which contact the ventricle with an apical process surrounded by ependymal cells forming pinwheel-like structures (Mirzadeh et al., 2008; **Figure 1**). They give rise to intermediate progenitors (transit-amplifying cells; Doetsch et al., 1999), the majority of which are actively cycling. These progenitors divide on average three times (during 3–4 days) before differentiating into neuroblasts, a half of which then divide at least once in the SVZ (Ponti et al., 2013). In most mammals, neuroblasts reach the olfactory bulb through "tangential chain migration," by sliding past each other in specific tunnels formed by an astrocytic meshwork (Lois et al., 1996; Peretto et al., 1997). About 10,000 new neurons are generated daily in the mouse SVZ (Ponti et al., 2013), half of which will die before functional integration (Petreanu and Alvarez-Buylla, 2002; Winner et al., 2002), the survivors differentiating into subsets of olfactory bulb interneurons (Obernier et al., 2014). Only small numbers of oligodendrocytes are generated *in vivo* (Menn et al., 2006), whereas in culture, after expansion of the NSC population, most of the progeny acquires aglial (mainly astrocytic) fate, with only 10–20% of neurons (Gritti et al., 2009).

In the SGZ, new neurons arise from two populations of primitive cells (radial – NSCs – and horizontal, slowly dividing cells; Ming and Song, 2011). Similarly to SVZ, they give rise to rapidly amplifying progenitor cells, which divide less than three times (Berg et al., 2015), and then in the next few weeks differentiate into immature neurons developing dendritic arborizations and axonal projections, then beginning to receive excitatory input from cortical perforant path axons (Vadodaria and Gage, 2014; Yu et al., 2014). Unlike SVZ neuroblasts, the hippocampal granule cell precursors perform a very short tangential and then radial migration, confined within the dentate gyrus.

The embryonic origin of the neurogenic niches is strictly linked to the proliferative activity of germinative layers, in periventricular position. The whole CNS forms by radial migration of the progeny from these layers, which mostly disappear postnatally. During development, the neurepithelium is in contact with both the ventricular and pial surfaces of the brain; then, as thickness increases, these cells transform into *radial glia*, a population of astrocytic precursors not only acting as scaffold for migrating neurons but also behaving as multipotent SCs (Malatesta et al., 2000; Noctor et al., 2001). Postnatally, quiescent radial glia-like cells persist as astrocytic-like SCs within remnants of the germinal layers (Tramontin et al., 2003; Merkle et al., 2004; Peretto et al., 2005; Yu et al., 2014; Nicola et al., 2015). In the SVZ, the SC process opposite to that "fishing" in the ventricle contacts the vasculature (Mirzadeh et al., 2008; **Figure 1**). Also, transit-amplifying cells directly contact blood vessels at specialized sites that lack glial and pericyte coverage (Shen et al., 2008; Tavazoie et al., 2008). Basal lamina structures extending from blood vessels to the ependymal layer do contact

TABLE 1 | Common features and differences between osteogenic and neurogenic niches.

	Osteogenic niche	Neurogenic niche
Types of niches	ALL BONES: *periosteal domain* (inner layer of the periosteum); *endosteal domain* (inner bone-lining and BM stroma) FLAT BONES OF THE SKULL: *suture domain* (within skull suture)	*V-SVZ* (lateral ventricle-olfactory bulb system) *SGZ* (hippocampus)
Number, location, distribution	*High number* (periosteal and endosteal niches are found in each skeletal bone); *Anatomically widespread* (in the whole bone)	*Very small number* (two main neurogenic sites in the brain) *Anatomically restricted* (ventricular lateral wall and dentate gyrus)
Types of stem cells (primary progenitors)	*Bone marrow stromal stem cells* (BMSCs; multipotent stromal, bone, cartilage, adipose, angiogenic progenitors) (αV integrin+, CD105+, STRO1+, CD45–; Tie2+; Nestin+) *Hematopoietic stem cells* (HSCs; multipotent blood cells and osteoclast progenitors) (CD45+, CD34+) (not considered here)	*Neural stem cells* (NSCs) *V-SVZ:* type B cells (radial glia-like cells, cilium) (Nestin+, GFAP+) astrocytic morphology *SGZ*: type 1 cells (radial glia-like cells) (Nestin+, GFAP+)
Number of stem cells	*High* (~12,000 clonogenicBMSCs through the skeleton of mice)	*Small* (~700 in the *V-SVZ*; the larger neurogenic site of mice)
Progeny and other niche contributors	PROGENY [Osteo-chondroblast lineage] *Skeletal stem cells* (SSCs; oligopotent – bone, cartilage, stromal progenitors; non-angio-, non-adipo-genic (CD105+, CD90+, Tie–); *Osteoblast progenitors* (CD90+, 6C3–, CD146+); *Osteoblasts* (metabolically active, OP+, OC+); *Osteocytes* (terminally differentiated, RANK L+ and ALP+) *Chondroblast/chondrocytes* (COL2+, ACAN+, SOX9+) [Osteoclast lineage] *Monocytes/macrophages* [from HSCs] (CD14+, CD33+) *Osteoclasts/osteoclast progenitors* (RANK+) LINEAGE SEQUENCE(S) Osteo/chondroblast lineage: *BMSCs* > *SSCs* > *osteoblast* or *chondroblast progenitors* > *osteoblasts* or *chondroblasts* > *osteocytes* or *chondrocytes* Osteoclast lineage: *HSCs* > *monocyte/macrophages* > *mononucleated osteoclast precursors* > *multinucleated mature osteoclasts* OTHER CONTRIBUTORS Stromal cells (6C3+; SDF1+), pericytes, e.c.m., endothelial cells, adipocytes, fibroblasts, nerve endings, dura mater (in skull bones)	PROGENY *V-SVZ*: *intermediate progenitor cells* (ASCLI+); migrating neuroblasts (PSA-NCAM+, DCX+) *SGZ*: *intermediate progenitor cells* (Type 2 cells) (Tbr2+, GFAP–, and mostly Nestin–) *Immature neurons* (*neuroblasts*) (PSA-NCAM+, DCX+) *Mature granule neurons* (functional nerve cells; NeuN+, Prox1+, DCX–) (also some *OPCs* and *mature oligodendrocytes*) LINEAGE SEQUENCE *Type 1 radial glia-like cells* (NSCs) > *intermediate progenitors* > *neuroblasts* > *immature neurons* > *mature neurons (some oligodendrocytes from OPCs)* OTHER CONTRIBUTORS Type 2 astrocytes (multipolar, GFAP+, S100β+, Nestin–), ependymal cells (in V-SVZ, facing the lateral ventricle), pericytes, endothelial cells, microglia, e.c.m. and fractones
Migration of the progeny	Osteoblasts, chondrocytes and osteoclastsdifferentiate locally, then migrate shortly during bone modeling/remodeling/healing Few osteoblast progenitors also migrate through blood circulation	*V-SVZ*: long distance migration in the olfactory bulb (mm in rodents; cm in primates); migration of OPCs into the white matter *SGZ*: short displacement within the dentate gyrus (up to hundreds μm)
Fate and final destination of the progeny	*Osteoblasts* at endosteum/periosteum-bone boundaries *Osteocytes* in interconnected lacunae (osteocyte syncytium) *Osteoclasts* in resorption (Howship's) lacunae *Chondrocytes* on articular surfaces, in cartilage molds-epiphyseal plates during endochondral ossification, in cartilaginous callus at fracture site	*V-SVZ*: *olfactory interneurons* (at least six different subtypes) in the olfactory bulb; some oligodendrocytes *SGZ*: *granule cells* (glutamatergic neurons) in the granule cell layer of the dentate gyrus
Origin	Periosteal/endosteal niches derive from embryo *mesoderm*: both BMSCs and HSCs come from *MPCs* Skull niches derive from neural crest (*neuroectoderm*): SSCs derive from *neural crest stem cells*	Niches derive directly (V-SVZ) or indirectly (SGZ) from the periventricular, embryonic germinal layers (*neuroectoderm*) NSCs come from embryonic *radial glia* (transient type of astrocytes)
Regulatory molecules/ pathways (in/on the niche)	Wnt/β-catenin, Ihh, FGF, IGF1, Twist1, RANK/RANKL/OPG, TGFβ, BMP-Smad, ERK, Ephrin, Kit-ligand, CXC-SDF, PTH/PTHrP, HIF1α, FoxC1, Heparanase, Kruppel-like factors 2 and 4, Hes4, Notch-Jag1 RunX2 common downstream transcription factor for most involved pathways Calcitonin, GH, PTH, PGE2, vit D3; sex hormones; cortisol; IGF; PDGF	*V-SVZ:* Wnt, BMPs, Noggin, IGF2, Shh (morphogens); EGF, FGF2, TGF-α, PDGF (growth factors); Notch, ASCLI/Mash1 (cell–cell interactions); GABA, Dopamine, Serotonin (neurotransmitters) Ephrines, ErbB4 and neuregulins *SGZ:* Wnts/sFRPs, IGF, BDNF, VEGF, EGF, IL4, IL6, IL1-β, TGF-β, TNF-α, GABA, Glu, dopamine, ACh, serotonin, leptin, estrogen, testosterone, corticosterone, endorphins
SC secretome [not considered here][a]	NGF, BDNF, GDNF;VEGF, VEGFR, IGF1-2, NT-3, NAP2b, FGF, PDGF, HGF, SDF-1, SCF; CXCRs; proteins and miRNA (in microvescicles)	NGF, BDNF, GDNF;CNTF, NT-3, VEGF, FGFII, PDGF; proteins and miRNA (in microvescicles)

(Continued)

TABLE 1 | Continued

	Osteogenic niche	Neurogenic niche
Relation/crosstalk with blood vessels	Perivascular localization of BMSCs, SSCs, osteoblast progenitors; IGF1, VEGF, PEDF, SDF1	Stem cells and transit-amplifying cells directly contact blood vessels; BDNF, IGF1, VEGF, PEDF, SDF1 (endothelial signals)
Rate of cell proliferation and progeny production	(Mouse endosteal niche) ~80% of endosteal BMSCs are clonogenic ~50% of endosteal osteoblasts are replaced over 14 days >80% of mature osteoblasts are replaced over 30 days	Less than 10% of Type 1 astrocytes (NSCs) proliferate ~87% of intermediate progenitors are actively cycling; they divide on average 3 times before differentiating; neuroblasts divide at least once ~10,000 new neurons are generated daily (mouse V-SVZ)
Homeostatic cell renewal	Rapid replacement of osteoblasts and osteoclasts *throughout the skeleton*, for bone modeling and remodeling (especially in periosteal domain); more active in long bones; limited in cartilage tissue	Neuronal replacement/addition *only within specific brain regions* (olfactory bulb and dentate gyrus); most of the CNS parenchyma is made up of non-renewing elements (apart from slow glial cell turnover)
Function of the finally differentiated cells	Matrix apposition (osteoblasts); mechano/chemo-sensing (osteocytes); bone resorption (osteoclasts); production of cartilage e.c.m. (chondroblasts/ chondrocytes)	Learning, memory (V-SVZ, SGZ) Pattern separation (SGZ) (partially still unknown)
Modulation of activity by environment	Physical activity, mechanical loading, trauma (stimulatory) [for internal regulation (e.g., hormones, growth factors) see above]	Physical activity (stimulatory); running: >neuronal production environmental enrichment: >integration; stress, aging (inhibitory); [for internal regulation (e.g., hormones, growth factors) see above]
Changes in activity with age	BMSC division decreases in terminally formed vs. developing bones, then decreases in elderly (disappears in suture domain) *Endosteal niche*: active during bone elongation *Periosteal niche*: rapid expansion at puberty (sexually dimorphic); slowly decreasing activity upon completion of longitudinal growth	*Rodents*: slow decrease of neurogenesis with age *Humans*: dramatic postnatal decrease in SVZ cell production and delivery to the olfactory bulb; substantial stabilization in SGZ
Reparative/ regenerative capacity	*Extensive* in fracture healing driven by the periosteal niche	*Limited* to the neurogenic sites and their tissue targets Largely *absent* in most CNS parenchyma
Inter-species differences	*Significant changes across vertebrate phylogeny; mostly conserved niche structure/functions* across most mammals; different chronology of niche activation and cell growth kinetics depending on animals' lifespan.	*Progressive reductionin spatial distribution and activity* from non-mammalian vertebrates to mammals; *V-SVZ*: early reduction in humans); *SGZ*: relatively constant through species
Stem cell behavior *in vivo* vs. *in vitro*	*Great differences* between *in vitro* and *in vivo* plasticity BMSCs and SSCs are easily isolated *in vitro*, highly expandable, multilineage potential. Extensive (though controversial) trans-differentiation potential; exclusive osteolineage fate *in vivo*	*Great differences* in differentiative fate between *in vitro* (multipotent) and *in vivo* (mainly neuronal) conditions Isolation through neurospheres (V-SVZ) or monolayer (SGZ) of highly expandable primary progenitors

Dashed areas refer to parameters which strongly (dark gray) or slightly (light gray) differ between the two systems.
V-SVZ, ventricular-subventricular zone; SGZ, subgranular zone; BM, bone marrow; e.c.m., extracellular matrix;COL2, type II collagen; ACAN, aggrecan; OP, osteopontin; OC, osteocalcin; ON, osteonectin; MPCs, mesodermal progenitor cells; GFAP, glial fibrillary acidic protein; DCX, doublecortin; PSA-NCAM, polysialylated form of the neural cell adhesion molecule; OPCs, oligodendrocyte precursor cells.
Note: the table content is referred only to non-hematopoietic cell components of the bone marrow niche, which are those involved in the formation of most bone precursors and stromal cells, and only indirectly involved in hematopoiesis, by supporting HSC homeostasis and maintenance.
[a]*For thorough discussion of MSC and NSC secretome, see Salgado et al. (2015) and Drago et al. (2013).*

cells at each stage of the lineage, binding growth factors (Mercier et al., 2002). In the SGZ, angiogenesis accompanies neurogenesis (Palmer et al., 2000), whereas the vascular bed is largely quiescent in SVZ. SC activity in the neurogenic niches is finely regulated by various signals involving growth factors, morphogens, cell–cell interactions, neurotransmitters, and endothelial signals (Tong and Alvarez-Buylla, 2014; **Table 1**). The whole process, from SC proliferation to neuronal integration, can be modulated by internal (hormones, trophic factors) and external (environmental) stimuli.

Both mammalian neurogenic niches show differences related to species and ages (Bonfanti and Peretto, 2011). The rostral migratory stream is active throughout life in rodents but temporally restricted to the first 18 months in humans (Sanai et al., 2011; Wang et al., 2011). By contrast, postnatal neurogenesis occurring in transient germinal layers of the cerebellum does persist in adult rabbits (Ponti et al., 2008). Unlike mammals, in which adult neurogenesis occurs mostly within two "canonical" neurogenic zones, in non-mammalian vertebrates NSCs and neurogenesis are widespread through many CNS regions (Zupanc, 2006; Grandel and Brand, 2013). During the last few years, new examples of cell genesis, involving neurogenesis and gliogenesis, have been shown to occur in adult parenchymal regions of the mammalian CNS (Bonfanti, 2013; Feliciano et al., 2015), where dividing progenitors have been detected, suggesting that *de novo* neural cell genesis could be more widespread than

previously thought (Nishiyama et al., 2009; Migaud et al., 2010; Bonfanti and Peretto, 2011). Yet, in most cases of parenchymal neurogenesis, the newly generated cells live only transiently and do not integrate in neural circuits, their role remaining obscure (Bonfanti and Peretto, 2011; Feliciano et al., 2015). Taken together, the highly restricted localization of adult neurogenesis in mammals underlines its exceptional character with respect to the genetically determined connectivity typical of most CNS tissue, which remains substantially refractory to cell renewal and regeneration.

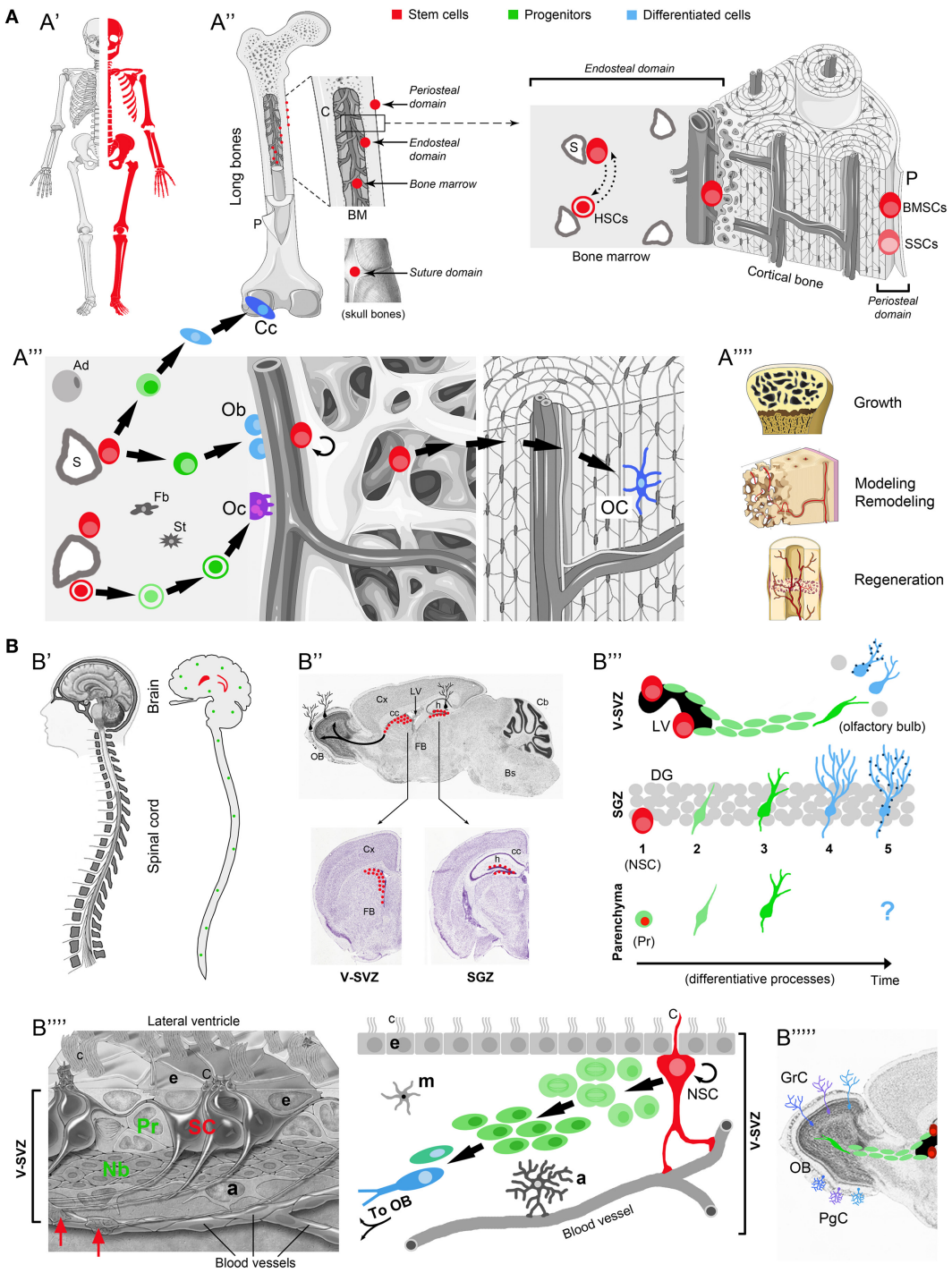

FIGURE 1 | Comparison between osteogenic and neurogenic niches.

(Continued)

FIGURE 1 | Continued
Localization and distribution in the body (A′-B′); localization and distribution in the organ (A′′-B′′); niche components and their reciprocal relationships (A′′′-B′′′′); final outcome in osteogenic/neurogenic (A′′′′-B′′′′′) and growth/regenerative processes (A′′′′). **(A)** Osteogenic niche. A′, All skeletal bones contain osteogenic niches through most of their extension; A′′, in most bones these niches can be found in periosteal, endosteal, and bone marrow (BM) position; in the skull, they occupy the suture domains; P, periosteum; BMSCs, bone marrow stromal cells; SSCs, skeletal stem cells; HSCs, hematopoietic stem cells; S, sinusoids; dotted lines with head arrows indicate reciprocal influence between BMSCs and HSCs. A′′′, Histological organization, cell components, lineage, and cell interactions in the osteogenic niche (endosteal domain); Ob, osteoblasts; Cc, chondrocytes; green cells: intermediate progenitors (osteoblast, chondroblasts, osteoclast, progenitors, macrophages); Oc, osteoclasts; OC, osteocytes; Ad, adipocytes; St, stromal cells; Fb, fibroblasts. A′′′′, Different outcomes from osteogenic stem cells involve both homeostatic cell renewal and lesion-induced regeneration (modified from "Slide kit Servier Medical Art," www.servier.com). **(B)** Neurogenic niche. B′ Two canonical neurogenic niches do contain stem cells in the brain (here represented in humans, their number and location being similar in mammals), and produce functional neurons for specific regions; parenchymal progenitors also divide throughout the CNS (green dots; not represented in B′′), yet giving rise to "incomplete" neurogenesis and gliogenesis (see B′′′). B′′, SVZ and SGZ niches on the wall of the lateral ventricles and in the dentate gyrus of the hippocampus (represented in mice; for differences in humans see **Table 1**); top, sagittal section; bottom, coronal sections; images from Allen Brain Atlas (Website: © 2015 Allen Institute for Brain Science. Allen Mouse Brain Atlas [Internet]; available from: http://mouse.brain-map.org.); Cx, cerebral cortex; cc, corpus callosum; OB, olfactory bulb; LV, lateral ventricle; h, hippocampus; Cb, cerebellum; FB, forebrain; Bs, brainstem. B′′′, Cell lineage and displacement; in canonical neurogenic sites (SVZ and SGZ) complete neurogenesis involves: dividing stem cells (SC) (1), secondary progenitor cells or neuroblasts (2), immature neurons (3), mature neurons (4), and fully integrated, functional neurons (5) (dark blue dots indicate the establishment of synaptic contacts). In non-canonical neurogenic sites (CNS parenchyma), only incomplete neurogenesis occurs, starting from parenchymal progenitors (Pr) and giving rise to a progeny of immature cells with apparently no further outcome [modified from Bonfanti and Peretto (2011)]. B′′′′, Left: histological organization of the SVZ neural stem cell niche; right: cell components, lineage, and cell interactions in the neurogenic niche. NSC, neural stem cell; Pr, progenitors (transit-amplifying cells); Nb, neuroblasts (forming chains which exit the SVZ by tangential migration); a, astrocytes; m, microglia; e, ependyma; c, cilia; C, radial glia-like cilium; red arrows, contacts between stem cell processes and blood vessels [modified from Mirzadeh et al. (2008)]. B′′′′′, Specific subpopulations of interneurons, e.g., granule cells (GrC) and periglomerular cells (PgC), functionally integrate in the olfactory bulb. Note the striking differences emerging in the two systems by comparing the extremes in **(A,B)** (A′ vs. B′, A′′′ vs. B′′′′′; see text).

SIMILARITIES AND DIFFERENCES BETWEEN OSTEOGENIC AND NEUROGENIC SYSTEMS

By comparing osteogenic and neurogenic SC niches a few similarities and significant differences emerge, concerning the relationships between SCs and the tissue/organ they belong to (**Table 1**; **Figure 1**). In both niches, close connections with blood vessels are observed, since blood-derived nourishment and signaling is vital to niche homeostasis. NSCs and BMSCs also share non-specific markers, such as the cytoskeletal protein nestin, a basic structural element in mitotically active cells, along with molecular signals which exert pleiotropic functions in development and homeostasis (e.g., Wnt, BMP, and Notch).

The most evident differences between osteogenic and neurogenic niches/systems are represented in the extremes of **Figures 1A,B**: abundant availability of widely distributed SCs/niches in bones (A′) grant continuous renewal and lesion-induced regeneration throughout the skeleton (A′′′), whereas highly restricted SCs/niches in the CNS (B′) only allow the renewal of well specified neuronal populations (B′′′′) (Obernier et al., 2014). In the whole mammalian body, the number and distribution of SC niches are highly heterogeneous, spanning from millions of "multiple, disperse" niches in blood, skin, and intestine (Nystul and Spradling, 2006), to only two niches capable of "complete" neurogenesis in the adult brain (Bonfanti and Peretto, 2011). These differences drive important consequences since multiple niches will allow homeostatic cell renewal and injury-induced regeneration in many tissues, whereas most brain regions are substantially non-renewing/non-regenerating (Bonfanti, 2011). Based on niche number, dislocation and rate of cell renewal, bone may be considered a borderland, given that osteogenic SCs are found throughout the skeleton. Accordingly, upon fracture, resident stromal, stem/progenitor cells, working in tandem with macrophages and circulating blood cells, lead to scarless healing (Colnot, 2009; Park et al., 2012). The mammalian CNS, in spite of its NSC content and intrinsic plasticity of neuronal and glial elements, shows very low and restricted rate of cell renewal, being hardly capable of repair from extensive damage or neuronal loss (Weil et al., 2008). NSC niches are deeply isolated within the most complex organ of the body, providing homeostatic replacement/addition of neurons only within restricted areas. Outside the neurogenic regions, in addition to the lack of SC niches, the substantial failure in CNS repair is due to evolutionary constraints, including incapability to recapitulate developmental pathways and strong immune reaction leading to necrosis instead of regeneration (Weil et al., 2008; Bonfanti, 2011). For these reasons, in spite of significant progress obtained in biomedical research, rational translation of the enormous body of basic research to the clinics is still very limited.

CELL–TISSUE SPECIFICITY AND TRANSLATIONAL ISSUES

It seems clear that SCs in the two niches originate from distinct embryo layers (except from skull SSCs), then adapt to utterly different morpho-functional environments: NSCs occupy topographically precise positions within specific neural systems, whereas BMSCs/SSCs, similarly to HSCs, balance free movement and stable positions. Hence, the general idea of using BMSCs as a regenerative treatment applied to CNS disorders is far from being substantiated. On the other hand, many studies support the evidence that BMSCs (as well as other MSC types) can produce beneficial – bystander – effects through the secretion of immune modulatory or neurotrophic paracrine factors (Martino et al., 2011; Drago et al., 2013). Nevertheless, the exact mechanisms underlying such effects are still far from being fully elucidated. Phase I–II clinical trials for neurological disorders (multiple sclerosis, amyothrophic lateral sclerosis, and spinal cord injury) suggested that autologous BMSCs inoculation is

safe and feasible and may induce systemic immunomodulatory effects explaining moderate clinical improvements. Conversely, no clear sign of neurodegeneration induced by cell replacement mechanisms could be investigated in any case, to date (Squillaro et al., 2015).

In addition, the heterogeneity of the BM stroma, in terms of cellular composition, is often neglected in the design of experimental cellular treatments, especially when minimal tissue manipulation (i.e., harvesting/fractionation and direct implantation, without prior culture amplification) is performed. It is worth noting that BMSC implantation experiments clearly indicated that the range of tissues which can be actually generated *in vivo* from both SSCs and BMSCs exclusively involves those making up the skeleton (Sacchetti et al., 2007; Bianco et al., 2013a,b; Tasso et al., 2013). Hence, the realistic translational consequences of *in vitro* BMSC plasticity are more limited than supposed, while their plausible trophic effect, in selected tissue environments, may be due to the innate role of BMSCs (and other MSCs) in forming supporting stroma in mesodermal-derived tissues, and to their rich secretome, exerting autocrine and paracrine effects (Kim et al., 2013; Tran and Damaser, 2015). On these bases, future studies should be aimed first at obtaining full understanding of the "natural" SC niche dynamics (paying attention to differences between tissues and species) and, second, at further elucidating the nature of cell-to-cell and molecular interactions adopted by different types of SCs in each physiological/pathological environment, allowing possible therapeutic outcomes.

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REVIEW
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Mesenchymal stem cells secretome as a modulator of the neurogenic niche: basic insights and therapeutic opportunities

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Neural stem cells (NSCs) and mesenchymal stem cells (MSCs) share few characteristics apart from self-renewal and multipotency. In fact, the neurogenic and osteogenic stem cell niches derive from two distinct embryonary structures; while the later originates from the mesoderm, as all the connective tissues do, the first derives from the ectoderm. Therefore, it is highly unlikely that stem cells isolated from one niche could form terminally differentiated cells from the other. Additionally, these two niches are associated to tissues/systems (e.g., bone and central nervous system) that have markedly different needs and display diverse functions within the human body. Nevertheless they do share common features. For instance, the differentiation of both NSCs and MSCs is intimately associated with the bone morphogenetic protein family. Moreover, both NSCs and MSCs secrete a panel of common growth factors, such as nerve growth factor (NGF), glial derived neurotrophic factor (GDNF), and brain derived neurotrophic factor (BDNF), among others. But it is not the features they share but the interaction between them that seem most important, and worth exploring; namely, it has already been shown that there are mutually beneficially effects when these cell types are co-cultured *in vitro*. In fact the use of MSCs, and their secretome, become a strong candidate to be used as a therapeutic tool for CNS applications, namely by triggering the endogenous proliferation and differentiation of neural progenitors, among other mechanisms. Quite interestingly it was recently revealed that MSCs could be found in the human brain, in the vicinity of capillaries. In the present review we highlight how MSCs and NSCs in the neurogenic niches interact. Furthermore, we propose directions on this field and explore the future therapeutic possibilities that may arise from the combination/interaction of MSCs and NSCs.

Keywords: mesenchymal stem cells, neural stem cells, niche, neurogenesis, secretome, regenerative medicine, interactions

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Introduction

Injury and disease within the central nervous system (CNS) frequently induce chronic and acute insults leading to irreversible processes of neuronal cell death. Understanding how neurogenesis can be modulated, either through drugs or interaction with other cell types, and neural progenitors recruited to the site of injury, is of the utmost

importance for the development of novel strategies that may impact the current state of the art. In recent years it has become evident that a population with a non-neural phenotype known for their role in the osteogenic niche, mesenchymal stem cells (MSCs), is able to regulate important phenomena within the CNS, including neural progenitor cells proliferation and differentiation. This quite unexpected and surprising function of MSCs brought closer the neurogenic and osteogenic niches, and prompted a new field of research that aims at understanding their interaction, and how both may impact on CNS regenerative medicine as we know it. Having this in mind the objective of the present paper is to review the most relevant advances in this field. It will first give an overview of neurogenic niches and how neurogenesis is regulated within them, then give an introduction to the osteogenic niches and MSCs, and end with a review on the most important works on the interactions between MSCs, neurogenic niches and disease models within the CNS.

Neurogenesis in the Adult Brain

Neuroanatomists have long believed Cajal's assumptions on the immutability of the CNS. This dogma has been challenged due to growing evidence that endow the brain with considerable regenerative potential and neuroplastic capacity, essential to promote brain homeostasis (Lemaire et al., 2012). It is now well established that adult neurogenesis occurs throughout life in specific brain regions where neurons are constantly generated (Doetsch et al., 1999; Gage, 2002).

Globally, this neuroadaptative phenomenon occurs by the re-organization of the neuromorphological and electrophysiological properties of post-mitotic cells and the generation of new neuronal or glial cells that will incorporate the pre-existing networks, a process therefore called neuro- or gliogenesis, respectively (Guan et al., 2009). This complex process involves several steps beyond cell division; these include the commitment of the new cell to a neuronal phenotype, the migration and morphophysiological maturation of the neuroblasts, and the establishment of appropriate synaptic contacts that culminate with a full integration on the pre-existent network. These spatially defined brain regions where neurogenesis occurs display a permissive microenvironment for maintenance, proliferation and differentiation of Neural stem cells (NSCs). Admittedly, at least two defined neurogenic brain regions are broadly recognized in the adult mammalian brain (**Figure 1**): the subependymal zone (SEZ) of the lateral ventricles, and the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG; Zhao et al., 2008). In both regions, astroglial cells act as the source of adult progenitor cells (Seri et al., 2001).

In the adult hippocampal neurogenic region, the progenitor cells reside in the SGZ, with defined gradients (Silva et al., 2006). Newly-born cells generated in the SGZ, become committed to a neuronal lineage and migrate into the granular cell layer (GCL), where they mature to become glutamatergic granule neurons (Seri et al., 2004; Zhao et al., 2008; Brill et al., 2009). The neuroblasts born in the SEZ migrate anteriorly along the rostral migratory stream (RMS), becoming mostly mature GABAergic granule neurons and periglomerular interneurons in the olfactory bulb (OB; Chumley et al., 2007; Zhao et al., 2008). Besides these two well-accepted adult neurogenic regions, although disputable, some reports have shown evidences for the generation of new neurons on other brain regions of the adult brain, including the amygdala (Bernier et al., 2002; Fowler et al., 2005; Gonçalves et al., 2008), the hypothalamus (Fowler et al., 2002; Kokoeva et al., 2005), the cortex (Gould et al., 1999; Kodama et al., 2004), the striatum (Dayer et al., 2005; Bédard et al., 2006) and the substantia nigra (SN; Zhao et al., 2003; Yoshimi et al., 2005). Importantly, it appears that neurogenesis in these regions occurs at very low levels or under non-physiological conditions (von Bohlen und Halbach, 2011).

Importantly, the neurogenesis process in the adult brain constitutes a new dimension of plasticity, with great impact on neuronal remodeling and repair, being now considered by the biomedical field as a promising therapeutical target in several neuropathological contexts. For instance, abnormal alterations in the hippocampal neurogenesis process have been implicated in an assortment of neuropsychiatric disorders (Sapolsky, 2000; Eisch et al., 2008; Kobayashi, 2009). Indeed, impairments in neuroplasticity are increasingly considered central to the ethiopathogenesis of depression (Bessa et al., 2009; Mateus-Pinheiro et al., 2013a,b). Studies have also shown the contribution of new neurons to a subset of hippocampal functions, influencing mood control, learning and memory (Hanson et al., 2011; Eisch and Petrik, 2012; Konefal et al., 2013). In fact, a clear connection between adult neurogenesis and learning/memory was demonstrated, as diminished neurogenesis decreases learning/memory, while enhanced neurogenesis improves it (Eisch and Petrik, 2012; Nakashiba et al., 2012). These examples prompt for the relevance of modulating the neurogenic niches as a potential therapeutic strategy to treat the symptoms of neurodegenerative disorders such as Parkinson's disease (PD), which we will later develop in the context of MSCs derived therapies.

We will next refer to the structural and functional organization specificities of the adult SGZ and SEZ neurogenic niches.

Adult Hippocampal Neurogenesis

As referred above, the adult brain is capable of generating new cells that can incorporate into its established complex circuitry (Trujillo et al., 2009). This process of adult neurogenesis highly recapitulates the embryonic neurogenic process, with the important difference that new neurons are generated in an already mature microenvironment and have to integrate in pre-existing neural circuits. Adult hippocampal neurogenesis consists of several highly regulated sequential phases (Kempermann et al., 2004; Ming and Song, 2005) characterized by morphological distinct cells: (i) proliferation of neural progenitor cells residing in a narrow layer of about three nuclei wide, the SGZ; (ii) generation of amplifying progenitors; (iii) cell migration; (iv) differentiation; and (v) maturation at the final destination with axon and dendrites formation and establishment of new synapses

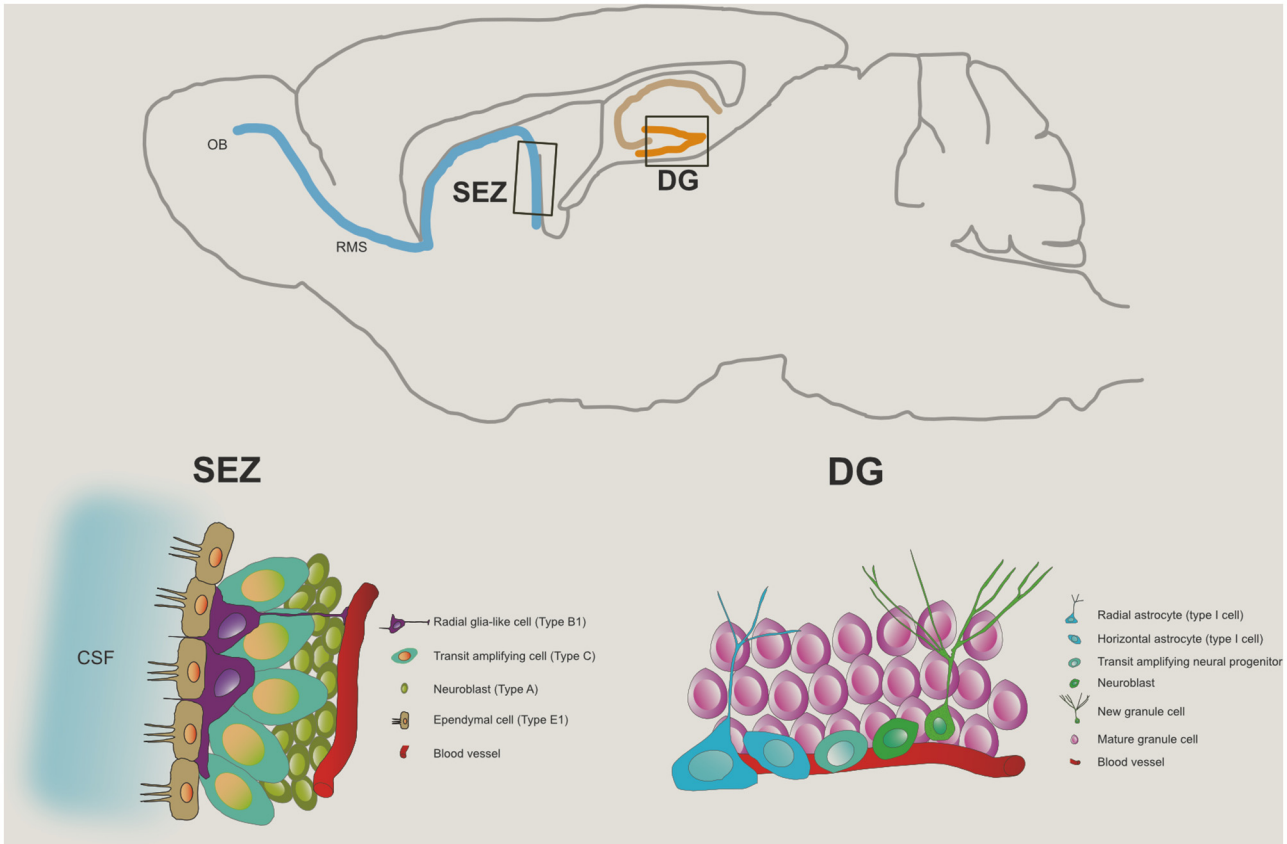

FIGURE 1 | Neurogenic niches in the adult brain. The top panel represents, in a saggital section of the rodent brain, the two major niches of neural progenitor cells in the adult brain: one, in the sub granular zone of the dentate gyrus (DG) of the hippocampus, and the other, the subependymal zone (SEZ), from where progenitor cells committed to the neuronal lineage migrate via the rostral migratory stream (RMS) towards the olfactory bulb (OB). The bottom left panel illustrates the typical cytoarchitecture of the SEZ niche while the cell population in the DG niche is presented in the bottom right panel.

(Kempermann et al., 2004; Steiner et al., 2006; Balu and Lucki, 2009).

The adult SGZ contains heterogeneous progenitor cells, which can be distinguished and identified by a particular set of molecules expressed by each progenitor population. The first type of progenitors are the quiescent neural progenitors (QNPs), described to be multipotent stem cells (Seri et al., 2001, 2004) and also known as NSCs or type-1 progenitor cells (Type-1 cells). These cells have morphological and antigenic glial properties, expressing markers such as the intermediate filament protein nestin, brain lipid binding-protein (BLPB), the glutamate aspartate transporter (GLAST; Steiner et al., 2006) and glial fibrillary acidic protein (GFAP), among others; it can be further distinguishable into two subtypes, based on their spatial orientation in the SGZ: radial astrocytes (rA) and horizontal astrocytes (hA). Radial astrocytes are characterized by having a single radial process, being also slowly dividing cells, whereas hA present a short horizontal process and divide faster (Lugert et al., 2010; Hodge et al., 2012). These cells divide asymmetrically giving rise to transient amplifying neural progenitors (tANPs, also designated as type-2 progenitor cells or TAPs). It is important to notice that this phase of the neurogenic process comprises a decisive point in the determination of neural progenitors cell-fate (neuronal or non-neuronal lineage commitment; Steiner et al., 2006). This latter progenitor cells, TAPs, are already committed to a neuronal lineage, being mitotically active (Encinas et al., 2006) and dividing symmetrically to give rise to neuroblasts (also known as type-3 cells). Neuroblasts are intermediate progenitors in the generation of new glutamatergic granule neurons, corresponding to a stage of transition from a slowly proliferating neuroblast, which is exiting the cell cycle, to a postmitotic immature neuron, that will migrate into the GCL of the DG. These neuroblasts express markers of the neuronal lineage, such as the polysialylated-neural cell adhesion molecule (PSA-NCAM), calcium-binding protein calretinin and doublecortin (DCX), that are crucial for further maturation and migration of these cells (Pleasure et al., 2000; Ehninger and Kempermann, 2003; Balu and Lucki, 2009). When reaching the GCL, newborn cells will fully maturate, elongating their axons towards the CA3 region (von Bohlen und Halbach, 2011) and establishing new functional connections (Balu and Lucki, 2009), thus becoming mature granule neurons, which express neuronal nuclei protein (NeuN). The cell markers

described above are not all exclusive to the SGZ; as will be described next, some are also characteristic of cells from the SEZ niche (**Table 1**). Moreover, similarly to the SEZ, only some of these markers allow cell-specific phenotypic characterization, as indicated in **Table 1**. Approximately 2–3 weeks after exiting the cell cycle, they express calbindin, a marker of mature granule cells (Kempermann et al., 2004). Newly formed neurons enter a period of enhanced synaptic plasticity in which their electrophysiological properties resemble those of neurons in the early postnatal period in juvenile animals (Ge et al., 2007). This phase lasts around 4–6 weeks after the original cell division, resulting in a total of approximately 7–8 weeks required for newborn cells to become functionally indistinguishable from the older granule cell population (Carlén et al., 2002; Abrous et al., 2005; Zhao et al., 2008; Snyder et al., 2009; Hanson et al., 2011). Newborn neurons display very different characteristics than mature ones, such as enhanced excitability, reduced threshold to induction of long-term potentiation (LTP) and an excitatory response to GABAergic input, since this neurotransmitter induces depolarization instead of hyperpolarization that is seen in adult neurons, which is related to a specific pattern of expression of some ionic co-transporters. This clearly indicates that adult-born neurons possess specific properties associated with plasticity (Schmidt-Hieber et al., 2004; Saxe et al., 2006; Ge et al., 2007; Hanson et al., 2011).

Noticeably, neurogenesis is a fine tuned process, in which not all cells expressing immature neuronal markers develop into fully mature neurons (Kempermann et al., 2003) and most newly-born neurons are eliminated by apoptosis (Biebl et al., 2000). The mechanisms that regulate this clearance of neurons are still to be fully understood, however, very recently a report showed that DCX-neuronal progenitors present phagocytic activity in the hippocampal and SEZ neurogenic niches and have great impact in the neurogenic process (Lu et al., 2011).

The harmonization of the several processes and cellular activities that occurs during the generation of new neurons in the adult mammalian brain is thus paramount. Several studies propose a complex transcriptional and epigenetic orchestration of the adult hippocampal neurogenic process, with both intrinsic and extrinsic factors being ultimately responsible for the modulation of this phenomenon. Therefore, the niche, where adult neurogenesis occurs is also crucial for the modulation and fine-tuning of this process.

Subependymal Zone Neurogenesis

The SEZ, also referred in the literature as adult subventricular zone (SVZ), is the site of the adult brain where neurogenesis is most intense. In rodents, the SEZ is seldom described as a thin layer of cells located below the ependymal layer that lines the lateral walls of the lateral ventricles, but it also extends to the dorsal and medial ventricular walls (Alvarez-Buylla et al., 2008). As in the SGZ niche, the cell populations in the SEZ are heterogeneous, containing several cell types that are identifiable by cell-specific markers. In general terms it might be described as being composed of slow-dividing type B cells (the NSCs) that originate fast-dividing type C cells, that in turn give rise to neuroblasts (type A cells). Nevertheless, given the complexity of these cell populations they, and respective phenotypic markers, will next be described with further detail (see also **Table 1**).

Type B cells are astrocytic cells and express the intermediate filament GFAP. In the SEZ two types of GFAP positive cells were distinguished according to ultrastructural differences: type B2 astrocytes, or niche astrocytes, display a highly branched morphology and are frequently found in the interface of the SEZ and the striatum (Doetsch et al., 1997); type B1 astrocytes are radial-glia like that organize in pinwheel structures with the apical ending, the primary cilium, turned towards the brain ventricles—and hence in bathed in the cerebrospinal fluid—and is surrounded by ependymal cells (Mirzadeh et al., 2008). The type B1 cells are recognized as the NSCs of the SEZ. Type C cells, or TAPs, originate from the NSCs. These rapidly dividing cells are organized in clusters of immature precursors that express distal-less homeobox 2 (Dlx2), achaete-scute complex homolog 1 (Ascl1or Mash1) and epidermal growth factor

TABLE 1 | Summary of markers that specifically allow phenotypic characterization of major cell types found in both neurogenic and osteogenic niches.

		Type-1 (NSCs)	Type-2 (TAPs)	Type-3 (Neuroblasts)	Mature neurons
Neurogenic niches	**SGZ**	GFAP GLAST	Mash1 Tbr2 Ngn2	DCX PSA-NCAM	NeuN
		Type B (NSCs)	**Type C (TAPs)**	**Type A (Neuroblasts)**	**Mature neurons**
	SEZ	GFAP GLAST	Mash1 Dlx2	DCX PSA-NCAM	NeuN Calretinin Calbidin GAD65 TH
Osteogenic niches	**Osteoblasts**	Runx-2; OCN; OPN; ON; ALP			
	MSCs	Positive for CD105, CD73, CD90 Negative for CD45, CD34, CD14, CD11b, CD79a, CD19			

SGZ, subgranular zone; SEZ, subependymal zone; NSCs, neural stem cells; TAPs, transient amplifying progenitors; GFAP, glial fibrillary acidic protein; GLAST, glutamate aspartate transporter; Mash1, mammalian achaete-scute complex homolog 1; Tbr2, T-box brain 2; Ngn2, neurogenin 2; DCX, doublecortin; PSA-NCAM, polysialylated-neural cell adhesion molecule; NeuN, neuronal nuclei; Dlx2, distal-less homeobox 2; GAD65, glutamate decarboxylase 65; TH, tyrosine hydroxylase; OCN, osteocalcin; OPN, osteopontin; ON, osteonectin; ALP, alkaline phosphatase.

receptor (EFGR; Ciccolini et al., 2005; Ming and Song, 2011). A short pulse (24 h) of the timidine analog BrdU mainly labels TAPs indicating that these cells are the largest pool of proliferating cells in the SEZ. Type A cells, or neuroblasts, are born from type C cells and constitute the neuronal precursors cells. Most type A cells express PSA-NCAM and DCX, which are associated to their migratory properties (Ming and Song, 2011). Under physiological conditions neuroblasts migrate tangentially from the SEZ, via the RMS to the OBs where they become fully mature neurons. Neuroblasts divide actively in the SEZ but also in the RMS. Once in the OBs, neuroblasts migrate radially, give rise to mature neurons and are integrated in distinct layers of the OB. They form new granular cells (deep, superficial and calretin positive) and periglomerular cells (calretin positive, calbidin positive and tyrosine hydroxylase positive; Lledo et al., 2008; Kriegstein and Alvarez-Buylla, 2009). Most of these new neurons are granule cells integrated in the granule cell layer and are GABAergic, but a small group of glutamatergic neurons was also identified (Brill et al., 2009).

Also of relevance in the SEZ are the ependymal cells (type E cells) that, as indicated above, form a monolayer that outlines the ventricular wall. These cells constitute a physical barrier that diminishes the direct and free exchange of molecules between the CSF and brain parenchyma (Falcão et al., 2012a). Two distinct ependymal cells have been described: the most common type E1 ependymal cells that are multiciliated, and the E2 ependymal cells that display two long cilia and represent solely 5% of the type E cells (Mirzadeh et al., 2008). Under physiological conditions these cells proliferate rarely (Coskun et al., 2008) or do not proliferate at all (Mirzadeh et al., 2008).

Tanycytes (Doetsch et al., 1997; Chojnacki et al., 2009), microglia (in response to injury; Ekdahl et al., 2009) and endothelial cells of the blood vessels (Tavazoie et al., 2008) are also relevant cellular components of the SEZ niche. These later cell types contribute to the specific microenvironment that constitute the SEZ NSCs niche; for instance, endothelial cells secrete several factors (pigment epithelium-derived factor, PEDF; NT3, among others) that induce proliferation and migration of NSCs (Ramírez-Castillejo et al., 2006; Delgado et al., 2014). Hence their interaction with proliferating cells should be taken into account when considering the modulation of the SEZ NSCs namely if one targets, for neuroregenerative purposes, the application of exogenous cells and/or protein/molecular factors, as will be further discussed in later sections.

In addition to the SEZ cellular heterogeneity, there is a further level of complexity in the form of topographical heterogeneity. A simple observation on the topography of the SEZ discloses major anatomical differences (Falcão et al., 2012b). It is now evident that even in the above described cell populations lays a remarkable heterogeneity either due to inherited intrinsic or epigenetic factors (Alvarez-Buylla et al., 2008) and/or an additional diversity in the surrounding microenvironment cues. Several studies showed that the NSCs pool is highly heterogeneous both in the origin and in cellular fate (Merkle et al., 2007; Alvarez-Buylla et al., 2008). For instance, while the common fate of SEZ born cells is the OB where they become interneurons, it was shown that it also generates a small pool of glutamatergic neurons steming from NSCs that reside in the adult dorsal wall of the lateral (Brill et al., 2009). Moreover, neuroblasts born either in ventral, dorsal, anterior or posterior regions are distinct, produce different neuronal types and are integrated in different layers of the OB (Alvarez-Buylla et al., 2008). As an example, neuroblasts from dorsal regions mostly originate superficial granule cells; while ventral derived neuroblasts give rise mostly to deep granule cells (Merkle et al., 2007). Also of notice, SEZ NSCs also originate oligodendrocyte precursors that migrate to the striatum and the corpus callosum and differentiate into oligodendrocytes (Nait-Oumesmar et al., 1999; Picard-Riera et al., 2002). The reason for why different regionally placed NSCs give rise to distinct progeny might reside in the distribution pattern of specific transcription factors, adding another layer of complexity in the regulation of cell proliferation in the SEZ, and thus in cell fate. All of these cell intrinsic and extrinsic aspects must be taken into account when considering putative therapeutic approaches for CNS regeneration.

Transcriptional Regulation of Adult Neurogenesis

Adult neurogenesis gives rise to both glutamatergic and GABAergic neurons. In the hippocampus changes in the rates of generation of glutamatergic neurons might contribute to several pathologies. In this context, the discovery of new factors important for the generation of glutamatergic neurons is needed. Interestingly, adult glutamatergic neurogenesis recapitulates the sequential expression of transcription factors found in the developing cerebral cortex (Pax6→Neurogenin2→Tbr2→Tbr1), demonstrating that this transcription network is maintained postnatally (Brill et al., 2009). For example, Pax6, a crucial determinant for the specification of glutamatergic neurons during development, is essential for adult neurogenesis (Hack et al., 2005) and is sufficient to instruct postnatal neocortical astrocytes towards neurogenesis *in vitro* (Heins et al., 2002). It was also shown during development, that one of the downstream targets of Pax6, the transcription factor AP2γ, is important for the specification of glutamatergic neocortical neurons and their progenitors (Pinto et al., 2009), and also for the differentiation of glutamatergic neurons in the adult neurogenic regions. Furthermore, AP2γ regulates Tbr2, which was shown to be important for glutamatergic neurogenesis during development (Pinto et al., 2009).

As described above, generation of specific cell types (neuronal or glial type) in the adult SEZ is topographically heterogeneous and this might be bound to transcriptional regulation. In fact, the expression of distinct transcription factors in both overlapping and non-overlapping regions of the SEZ is described. Similarly to the SGZ, some of these transcription factors were correlated with the SEZ embryonic origin (Waclaw et al., 2006; Young et al., 2007). In fact, a topographical pattern of transcription

factors expression in the SEZ is associated with NSCs embryonic origin and adult neuronal fate. Generally, NSCs in the lateral ventricular wall ubiquitously express Dlx1, 2, 5 and Mash1, while Emx1 expression is exclusive to the dorsal wall of the ventricle (Young et al., 2007). Furthermore, the transcription factors Nkx2.1 and Pax6 outline the ventral and dorsal regions of the lateral wall, respectively (Alvarez-Buylla et al., 2008; Weinandy et al., 2011). Thus, in the SEZ, an additional challenge is to understand how to modulate different combinations of transcription factors so as to result in production of specific neuronal types.

A targeted induction of neurogenesis, by stimulating endogenous neural progenitors in the adult brain, could represent an important cellular therapy to treat neurodegenerative disorders. A major challenge in our days is to improve survival and induce differentiation of newborn neurons after acute lesions. For instance, it was already shown that Pax6 can induce neurogenesis from non-neurogenic astrocytes *in vivo*, when overexpressed after stab-wound lesion (Buffo et al., 2005). These experiments provide proof of principle that neurons can be newly generated from endogenous sources of the adult mammalian brain. However, these induced neurons are very few in number and fail to mature. Therefore, new cues are needed to efficiently instruct neurogenesis and repair after neuronal insult.

The Microenvironment of the Neurogenic Niches

The interplay between extrinsic and intrinsic factors determines the NSCs niche homeostasis. Intrinsic factors are a set of signals produced by the progenitors that together with exterior microenvironment cues (extrinsic factors) instruct distinct neurogenic phases and ultimately the cellular fate. Many of the mechanisms regulating NSCs proliferation and neurogenesis during embryonic development, appear to be conserved in adulthood, and both intrinsic and extrinsic factors important for embryonic neurogenesis are also involved in the regulation of neurogenesis in the adult brain (Ming and Song, 2011). However, there are relevant differences between them, especially regarding the properties of the cellular and molecular niche. Whereas during development, the cellular environment is highly specialized to support proliferation, in the adult neurogenic niches the environmental context is concomitantly able to maintain a population of fully mature neurons (Zhao et al., 2008; Jessberger et al., 2009), thus providing a different set of both intrinsic and extrinsic signals.

Extrinsic signals, for instance, for the SEZ regulation include several trophic and growth factors, neurotransmitters, morphogens, hormones and cytokines (Falcão et al., 2012a). These extracellular signaling molecules are of diverse origins, namely from ependymal cells, endothelial cells, neural progenitor cells and neurons. The neurotransmitters are examples of key extrinsic factors of neuronal origin. For instance, the neurotransmitter GABA produced by niche neuroblasts is reported to inhibit NSCs proliferation but serotonine stimulates NSCs proliferation (Banasr et al., 2004, and conflicting results were presented for the effects of dopamine (DA) in the SEZ niche (Berg et al., 2013).

This important role of the microenvironment in the neurogenic niches for the regulation of NSCs has been shown by many *in vivo* and *in vitro* studies. For example, SEZ derived neuroblasts can change their fate and differentiate into oligodendrocytes upon a change in the microenvironment induced by demyelination of the corpus callosum (Picard-Riera et al., 2002; Jablonska et al., 2010). Additionally, glial progenitor cells may change to a neuronal fate when transplanted into a neurogenic region (Shihabuddin et al., 2000), while mouse SEZ neural progenitors committed to the neuronal lineage, changed to glial differentiation upon transplantation into regions outside the neurogenic niche (Seidenfaden et al., 2006).

The microenvironment of the neurogenic niches is thus essential for fate determination and cell differentiation, as well as for self-renewal, proliferation, migration and maturation of NSCs. This microenvironment is comprised of local cell types, cell signals, extracellular matrix and microvasculature. Indeed, the SEZ and SGZ niches are highly vascularized by a network of specialized capillaries (Goldberg and Hirschi, 2009) and NSCs closely interact with the microvasculature (Palmer et al., 2000; Mirzadeh et al., 2008; Shen et al., 2008; Tavazoie et al., 2008). This microvasculature has been shown to be essential in maintaining the function of the neurogenic niches, namely by regulating the proliferation and quiescence of NSCs (Palmer et al., 2000; Shen et al., 2004, 2008; Tavazoie et al., 2008; Culver et al., 2013), as well as NSCs self-renewal and neurogenesis through soluble factors secreted by the endothelial cells (Shen et al., 2004; Ramírez-Castillejo et al., 2006; Gómez-Gaviro et al., 2012). Noteworthy is the recent report of the existence of MSCs in the brain microvasculature (Paul et al., 2012), which paves way for the usage of MSCs secretome to modulate the neurogenic niches cells. One further example of NSCs microenvironment modulators are microglia cells, the brain resident macrophages, have also a crucial role in the regulation and maintenance of neurogenesis in the SGZ neurogenic niche (Sierra et al., 2010) given that they impact on the proliferation of neural stem/progenitor cells (Gebara et al., 2013); also they are particularly relevant in modulating the SEZ in response to brain injury (Thored et al., 2009).

In this way, signaling from and into the niche is suggested to be responsible for key processes in the regulation of homeostasis of adult neurogenesis including the balance between quiescence vs. proliferation, the mode of cell division, and the prevention of stem cell depletion.

The existence of NSCs in the adult neurogenic niches prompted research for their usage in adult brain regeneration. Nevertheless, their intrinsic and extrinsinc properties, which we have summarized above, pose also major challenges to mount adequate therapeutic approaches. MSCs, and specifically the interaction of their properties with NSCs, might be ideal candidates for this purpose. We will next describe the major characteristics of MSCs and how they might promote brain regeneration.

The Osteogenic Niche

The osteogenic niche is a highly vascularized and dynamic environment in which four cell types play an important role on the maintenance and renewal of bone tissue: MSCs, osteoblasts, osteocytes and osteoclasts.

Osteoblasts (**Table 1**) arise from osteoprogenitor and MSCs (further details on MSCs biology are discussed in "MSCs and CNS Therapies" Section) present in the bone marrow and periosteum. They are known to be involved in the synthesis and regulation of extracellular matrix elaboration (ECM) and mineralization (Sommerfeldt and Rubin, 2001; Salgado et al., 2004). Furthermore, it is also known that basic cellular functions and responsiveness to metabolic and mechanical stimuli demand are maintained through extensive cell-matrix and cell-cell contacts via a variety of transmembranous proteins and specific receptors (Sommerfeldt and Rubin, 2001). Osteocytes represent osteoblasts that became incorporated in the newly elaborated extracellular matrix, being enclosed in spaces called lacunae. They maintain direct contact with neighboring osteocytes, osteoblasts and bone lining cells through cellular processes that are created before and during matrix synthesis (Sommerfeldt and Rubin, 2001; Knothe et al., 2004). In mature bone these cell processes are contained in channels called the canaliculi. The communication and interaction between neighboring osteocytes is achieved through the establishment of gap junctions (Sommerfeldt and Rubin, 2001; Knothe et al., 2004). This is an absolute need for osteocytes because is the only way by which they can assure the access to oxygen and nutrients. They are known to be involved in the calcification of osteoid matrix, blood-calcium homeostasis and to be the mechanosensor cells of bone (Sikavitsas et al., 2001; Knothe et al., 2004). Finally, **osteoclasts**, are multinucleated polarized cells involved in the bone remodeling process, that belong to the monocyte/macrophage lineage. Their main function is to resorb mineralised bone. For this purpose they are enriched in intracellular structures such as pleomorphic mithocondria, vacuoles, and lysossomes, as well as alterations, namely at the structural level, in its cell membrane (Vaananen, 1996).

Mesenchymal Stem Cells

Mesenchymal Stem Cells, The Secretome and Neurogenic Niches

The first reports on the possible existence of a population with a Mesenchymal progenitor character are attributed to Friedenstein et al. (1974b). Indeed, Friedenstein et al. identified and defined these cells as plastic-adherent fibroblast colony-forming units with clonogenic capacity (Friedenstein et al., 1974a). Later, these cells were also named as marrow "stromal cells", on the basis of their possible use as a feeder layer for hematopoietic stem cells (Eaves et al., 1991; Glavaski-Joksimovic and Bohn, 2013). Additionally other reports also referred to them as MSCs because of their clonogenicity capacity and ability to undergo multilineage differentiation (Caplan, 1991; Bluguermann et al., 2013). Currently MSCs have been defined, according with the International Society for Cellular Therapy (ISCT) criteria, as multipotent cells (with the ability of at least differentiating towards the osteogenic, chondrogenic and adipogenic lineages), capable of self-renewal, able to adhere to tissue culture plastic and to display the presence of surface markers (CD105, CD73, CD90), as well as the lack of hematopoietic cell surface markers (CD45, CD34, CD14 or CD11b, CD79a or CD19 and Human Leukocyte Antigen DR; **Table 1**; Dominici et al., 2006). So far, MSCs have been isolated from bone marrow (BMSCs), adipose tissue (ASCs), dental pulp, placenta, amniotic fluid, umbilical cord blood, umbilical cord Wharton's jelly (bulk—WJ-MSCs; perivascular region—human umbilical cord perivascular cells, HUCPVCs), liver, lung and spleen, and brain (for an extensive review see Teixeira et al., 2013). As potential therapeutic agents, MSCs display a number of key characteristics that are believed to be advantageous when compared to other cell populations. For instance they can be isolated with minimal invasive procedures, easily cultured and expanded *in vitro* for several passages, can be used for allogenous transplantation in virtue of their hypoimmunogenicity, decreased tumorigenic potential and, as adult cells, are not hindered by ethical concerns (Salgado et al., 2006; Kishk and Abokrysha, 2011; Seo and Cho, 2012; Teixeira et al., 2013). These MSCs features have made them attractive tools for CNS neurodegenerative diseases.

Initially it was considered that the true therapeutic potential of these cells relied on their multilineage differentiation. Indeed most of the literature of the 90 s and early 21st century was focused on the differentiation of these cells towards mesodermal lineages, such as the osteogenic, mainly within 3D matrices known as scaffolds, to induce regeneration in the affected areas. Around the same time it was also suggested that MSCs even had a greater differentiation potential than was originally predicted, as several reports indicated that these cells could be differentiated beyond the mesodermal lineages (Dominici et al., 2006). In 2005, Gnecchi et al. (2005) put forward a new concept that lately would change the paradigm of how MSCs could be used in regenerative medicine, by showing that their therapeutic potential was mostly related to the growth factors that they secreted to the extracellular milieu, rather than to their differentiation potential.

Indeed, in recent years it is becoming increasingly accepted that the regenerative effects promoted by MSCs are mainly associated with their secretome. As discussed by Teixeira et al. (Teixeira et al., 2013) the secretome of MSCs is composed by a proteic soluble fraction, constituted by growth factors and cytokines, and a vesicular fraction composed by microvesicles and exosomes, which are involved in the transference of proteins and genetic material (e.g., miRNA) to other cells. The protective actions promoted by MSCs secreted molecules are closely related with therapeutic plasticity in the CNS. Indeed several authors have reported the presence of a plethora of growth factors with a known influence on neuronal survival, differentiation, neurite outgrowth and immunomodulation of microglial cells; these factors are BDNF, glial derived neurotrophic factor (GDNF), nerve growth factor (NGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), VEGF-receptor 3 (VEGF-R3), angiopoietin 1, insulin-like growth factor

1 (IGF-1), insulin-like growth factor 2 (IGF-2), epidermal growth facto (EGF), basic fibroblast growth factor (bFGF), FGF 20, granulocyte colony-stimulating factor (G-CSF), platelet-derived growth factor AA (PDGF-AA), chemokine ligand 16 (CXCL 16), neutrophil-activating-protein-2 (NAP 2) and neurotrophin-3 (NT-3) growth factors, as well as interleukin-6 (IL-6), interleukin-10 (IL-10), transforming growth factor beta 1 (TGF β1), stem cell factor (SCF), stromal cell-derived factor 1 (SDF-1) and monocyte chemotactic protein 1 (MCP-1) cytokines (Rehman et al., 2004; Caplan and Dennis, 2006; Chen et al., 2008b; Bonfield et al., 2010; Meyerrose et al., 2010; Nakano et al., 2010; Ribeiro et al., 2012). Other proteins such as 14-3-3, ubiquitin carboxyl-terminal esterase L1 (UCHL1), hsp70 and peroxiredoxin-6 have also been related to the neuroregulatory character of the secretome of MSCs (Fraga et al., 2013).

The action of MSCs and their secretome in neurogenic niches (**Figure 2**) such as the SGZ has been previously described. For instance, Munoz et al. (2006) transplanted BMSCs into the DG of immunodeficient mice. Results revealed that the transplanted MSCs markedly increased the proliferation of endogenous NSCs that expressed the stem cell marker Sox2, as well as their differentiation, a fact that was attributed to a local increase on the expression of growth factors such as VEGF, ciliary neurotrophic factor (CNTF), neurotrophin-4/5 and NGF. More recently it was also shown that the injection of the secretome of MSCs itself, was also able to modulate both neuronal survival and differentiation within the adult rat hippocampus. Teixeira et al. (2015) show that the injection of the secretome of HUCPVCs (a MSC population that resides in the perivascular region of the umbilical cord) was able to induce an increased number of DCX^+ cells. This observation was then related with a higher expression of FGF-2 and NGF in the injected area.

As a consequence of this, the multiple faces of MSCs and their secretome have prompted a number of different experimental therapeutic strategies in CNS regenerative medicine. Such strategies rely on a strong interplay between neuroregulatory molecules secreted by MSCs and the different niches with the CNS.

In disorders such as multiple sclerosis (MS), available data, both from animal models and human patient related

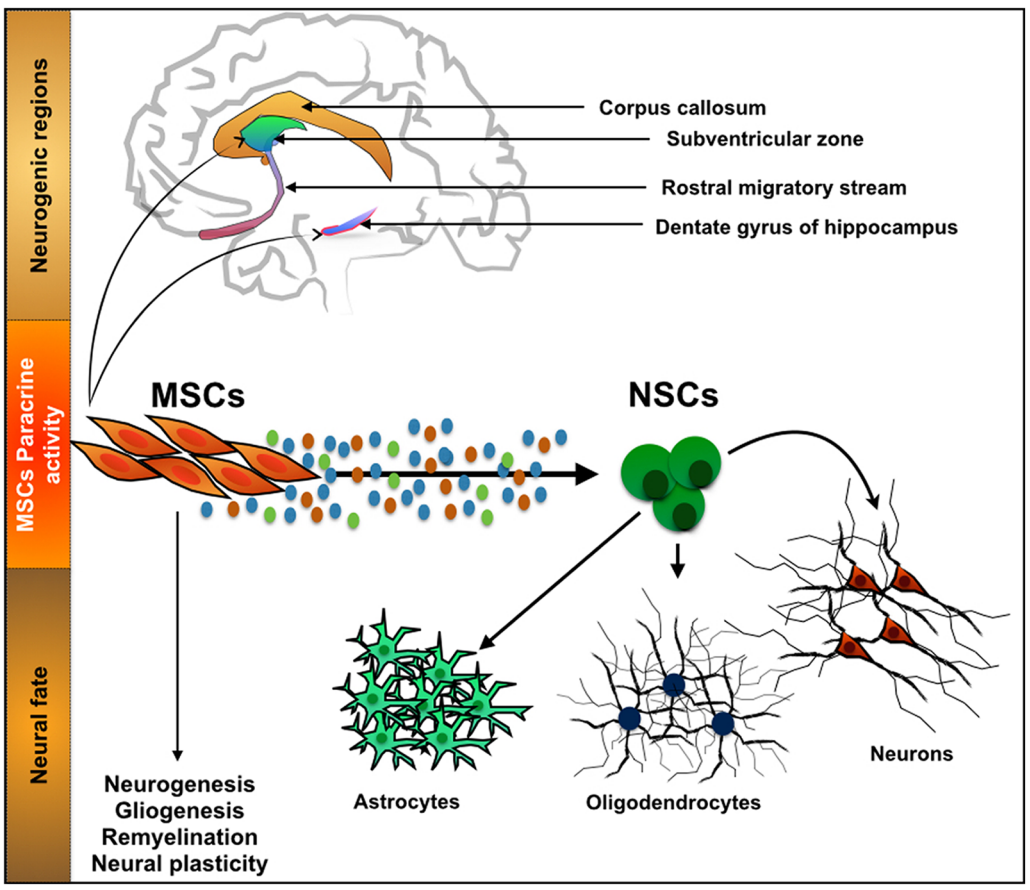

FIGURE 2 | Interaction between mesenchymal stem cells (MSCs) and neurogenic niches. MSCs, a cell population with a known function in the osteogenic niche, is able to modulate the action of Neural stem cells (NSCs) by means of their secretome. Through the secretion of neuroregulatory molecules, either soluble or in the form of vesicles, MSCs are able to influence processes such as neurogenesis, gliogenesis, remyelination and neural plasticity. With it important developments have been recently witnessed in CNS regenerative medicine strategies.

studies, indicates that the immunomodulatory properties of the secretome of MSCs regulate the immune/oligodendrogenic niches. For instance Wang et al. (2010) revealed that MSCs derived from human embryonic stem cells (hES-MSCs) significantly reduce clinical symptoms and prevent neuronal demyelination in a mouse experimental autoimmune encephalitis (EAE) mouse model of MS, by reducing the frequency of CD4+ and CD8+ cells infiltration in the CNS. A similar trend was described by Li et al. (2014) and Llufriu et al. (2014) in studies with human patients, in which the administration of MSCs from different sources, alone or combined with pharmacotherapies, positively impacted the condition of the patients, by modulating MS related inflammatory events.

On other hand, in disorders such as PD, Ischemic Stroke (IS) and Glioblastoma Multiforme (GBM) it is believed that the action of MSCs goes beyond the neuro-immunomodulation, and in fact, some of the reported benefits may be closely related with their direct interaction with the neurogenic niches. Due to the nature and objectives of this review, this topic will be further explored in the following section.

MSCs and CNS Therapies

Parkinson's Disease

Among CNS disorders, PD is the most common motor-related disorder in middle or late-life affecting millions (Pereira and Aziz, 2006) worldwide. It is a slowly progressive neurodegenerative disease that is primarily characterized by the loss of dopaminergic (DAergic) neurons in several dopaminergic networks, most intensively in the ventral tier of the substantia nigra *pars compacta* (SNpc) within the mesostriatal/nigrostriatal pathway (Koller, 2003; Pereira and Aziz, 2006; Cummins and Barker, 2012; Teixeira et al., 2013). The depletion of SN neurons leads to the loss of DAergic innervations and consequently to striatal DA deficiency, which is responsible for the major sensory-motor symptoms of PD (Dauer and Przedborski, 2003).

A considerable body of evidence has revealed the potential of MSCs to promote protection and/or recovery of DAergic neurons against neurotoxin-induced nigrostriatal degeneration. Indeed, several studies have demonstrated that BMSCs secretome protect and/or regenerate DAergic neurons in *in vitro* and *in vivo* models of PD, through the secretion of growth factors and cytokines (summarized in **Table 2**; Weiss et al., 2006; Shintani et al., 2007; McCoy et al., 2008; Kim et al., 2009; Sadan et al., 2009; Blandini et al., 2010; Cova et al., 2010; Wang et al., 2010; Danielyan et al., 2011; Park et al., 2012). For instance, Shintani and coworkers demonstrated that BMSCs conditioned media (CM) was able to promote survival of tyrosine hydroxylase (TH)-positive DAergic neurons in rat primary cultures of ventral mesencephalic cells (Shintani et al., 2007). Moreover, intrastriatal transplantation of fetal mesencephalic cells treated with human BMSCs CM, during steps of donor preparation and implantation, induced survival of DAergic grafted cells and promoted functional recovery in a 6-OHDA rat model of PD (Shintani et al., 2007). The observed protection of DAergic neurons was attributed to BMSCs secretion of BDNF, GDNF and bFGF. Similarly, Sadan et al. showed that human BMSCs (hBMSCs) cultured in the presence of growth factors, not only significantly increased the viability of the SH-SY5Y neuroblastoma cell line exposed to 6-OHDA, but also that BMSCs transplanted into the striatum of a 6-OHDA rat model of PD, migrated to the lesion site, and increased the numbers of TH-positive cells and DA levels (Sadan et al., 2009). These neuroprotective and neuro-regenerative effects were accompanied by an improvement in animals' motor behavior and were correlated with BMSCs secretion of BDNF and GDNF. This expression pattern is in accordance with data published by Blandini and co-workers using the same animal model (Blandini et al., 2010). On the other hand, Wang and colleagues associated rat-derived BMSCs expression of stromal cell-derived factor 1 (SDF)-1α with the DAergic neurons protection against 6-OHDA neurotoxin both *in vitro* and *in vivo*, through anti-apoptotic based mechanisms (Wang et al., 2010). Moreover, Cova et al.

TABLE 2 | Summary of the studies focused on the impact of MSCs on multiple aspects of PD regenerative medicine.

Reference	Outcomes
Weiss et al. (2006)	• Transplantation of MSCs isolated from the Wharton Jelly into a 6-OHDA rat model led to behavioral improvements and a local increase of GDNF.
Shintani et al. (2007)	• CM of BMSCs promoted survival of TH^+ neurons; • Transplantation of fetal mesencephalic cells treated with BMSCs CM promoted functional recovery in a 6-OHDA rat model.
Sadan et al. (2009)	• BMSCs transplantation into a 6-OHDA rat model led to increased TH+ cells and tissue DA levels; • Data correlated with secretion of GDNF by BMSCs.
Wang et al. (2010)	• BMSCs protected DA neuronal apoptotic cell death through SDF-1α.
Cova et al. (2010)	• Long term survival of BMSCs upon transplantation into the striatum; • Increased neurogenesis in SVZ; • Survival of DAergic terminal.
Danielyan et al. (2011)	• Intranasal delivery of BMSCs in a 6-OHDA rat model reduced the levels of pro-inflammatory cytokines.

OHDA, Hydroxidopamine; GDNF, Glial Derived Neurotrophic Factor; TH, Tyrosine Hydroxylase; CM, Conditioned Media; DA, Dopamine; SDF, Stromal Cell Derived Factor; SVZ, Subventricular Zone.

demonstrated that BMSCs transplanted in the striatum of a 6-OHDA rodent model of PD were able to survive and interact with the lesion site surroundings, thus enhancing the survival of DAergic terminals and neurogenesis in the SVZ in a sustained manner (Cova et al., 2010). Finally, the secretion of BDNF *in vivo* by BMSCs, was correlated with the activation of endogenous stem cells (Cova et al., 2010).

In addition to the capability of BMSCs to induce survival of DAergic neurons, its effects have also been related with their immunomodulatory properties. In this context, intranasally delivered rat BMSCs into 6-OHDA hemi-parkinsonian rats migrated toward the SN and the striatum and reduced the overall expression of pro-inflammatory cytokines, such as IL-1β, IL-2; IL-12; tumor necrosis factor alpha (TNF-α) and interferon γ (INF γ). Moreover, their presence also revert the loss of nigral DAergic neurons and striatal fibers (Danielyan et al., 2011).

From the above-referred studies, it is clear that there is increasing evidence indicating that the neuroprotective and neuroregenerative effects of MSCs observed in PD are attributed to the secretion of soluble growth factors and cytokines. The secretion of these factors by MSCs not only protects DAergic neurons from further degeneration and enhances endogenous restorative processes (e.g., neurogenesis), but also acts as inflammation and immune response modulators. Moreover, recent reports have shown that besides soluble growth factors and cytokines, MSCs also secrete microvesicles and exosomes containing mRNAs and/or miRNAs (microRNAs), which are believed to mediate cell-to-cell communication and act as reparative agents (Baglio et al., 2012). Indeed exosomes secreted by BMSCs *in vitro* not only mediate communication with neurons and astrocytes, but also regulate neurite outgrowth by transfer of miRNA (miR-133b) to neural cells (Xin et al., 2012).

Ischemic Stroke (IS)

Cerebrovascular diseases, such as stroke, result from blood vessel occlusion or damage, leading to focal tissue loss and death of endothelial cells and multiple neural populations (Lindvall and Björklund, 2004; Lindvall and Kokaia, 2010).

It has been proposed that the transplantation of MSCs (summarized in **Table 3**) can represent a feasible therapeutic option for IS (Locatelli et al., 2009). Indeed, studies have shown that after intravenous administration of BM-MSCs, these have the capacity to migrate to the lesion site promoting tissue regeneration and behavioral improvement (Komatsu et al., 2010). Moreover, these cells were able to promote neurogenesis, increase the survival of neuroblasts and to reduce the volume of lesion after IS (Keimpema et al., 2009; Zheng et al., 2010). According to Wakabayashi and colleagues the secretion of molecules such as IGF-1, VEGF, EGF, BNDF and bFGF mediate some of the observed effects, namely the reduction of lesion size and the modulation of the inflammatory environment for host cells (Wakabayashi et al., 2010). Leu et al. (2010) also proposed that like BM-MSCs, adipose stroma/stem cells (ASCs) therapy also enhances angiogenic and neurogenic processes. Although the exact mechanism of these cells remains still unclear, other studies have suggested that homing properties, cytokines (SDF-1α, IL-1, IL-8) effects, and paracrine mediators (HGF, BDNF, IGF-1, VEGF) could pinpoint ASCs effects, contributing to tissue regeneration and functional behavior (Tang et al., 2005; Banas et al., 2008; Chen et al., 2008a). On the other hand Koh et al. (2008) also demonstrated that MSCs exhibited a migratory tropism to the lesion site, which might foster the creation of new networks between the host neural and transplanted stem cells (Koh et al., 2008). Additionally exosomes secreted by MSCs were also shown to mediate important actions in these environments. Xin et al. (2013b) suggested that the observed improvements were due to the presence of miRNA-133b in the exosomal fraction of MSCs that were transplanted into a middle cerebral artery occlusion (MCAo) rat model. Similarly, the same authors also demonstrated that after systemic administration of MSCs-derived exosomes, there was an increase in neurovascular plasticity, which led to an enhancement of the functional recovery of an animal model of stroke (Xin et al., 2013a,b).

Glioblastoma Multiforme (GBM)

Malignant gliomas are particularly dramatic cancers of the CNS, ranking first among all human tumor types for tumor-related average years of life lost (Burnet et al., 2005). GBM is the most common and most malignant subtype (Ohgaki and Kleihues, 2007), typically treated with surgery, radiotherapy and temozolomide (TMZ)-based chemotherapy (Stupp et al., 2005). Despite this multimodal approach, virtually all GBMs eventually recur and are fatal. GBMs present critical hallmark features that largely contribute to treatment failure, including their high invasive capacity, the presence of the bood-brain barrier, and remarkable genetic and epigenetic heterogeneity. Additionally, GBMs present a small population of cells with neural stem cell-like properties (Singh et al., 2003), called glioma stem cells (GSC), which display remarkable features in the context of glioma pathophysiology, including self-renewal capacity (generating both GSCs and non-GSCs cancer cells necessary for tumor maintenance), multipotency (differentiating into diverse cell population lineages), and prominent tumorigenic potential *in vivo*. In resemblance with NSCs that are located in specific highly-vascularized neurogenic niches of the adult brain, GSCs also accumulate and depend on the prominent vasculature of these regions to control their stemness and differentiation processes (Folkins et al., 2007; Calabrese et al., 2007; Gilbertson and Rich, 2007; Hadjipanayis and van Meir, 2009). GSCs have been shown to be more resistant to radiation and conventional chemotherapeutic drugs, and are believed to be responsible for tumor relapse observed almost universally in GBM patients (Singh et al., 2003; Bao et al., 2006; Calabrese et al., 2007; Chalmers, 2007). Since the clinical prognosis of GBM patients has not improved significantly in the last years, it is urgent to develop novel unconventional therapeutic strategies.

Like in other cancer types, a relatively new and promising therapeutic approach to tackle malignant gliomas is based on the use of (normal) stem cells. The most unique and critical

TABLE 3 | Impact of MSCs administration on ischemic stroke related animal models.

Reference	Outcomes
Koh et al. (2008)	• MSCs exhibited migratory tropism to injury sites.
Komatsu et al. (2010)	• Intravenous delivery of MSCs promoted tissue regeneration and behavioral improvement.
Keimpema et al. (2009); Zheng et al., 2010	• Reduction of the volume of the injury after IS; • Increased levels of neurogenesis; • Survival of neuroblasts.
Wakabayashi et al. (2010)	• Reduction of the injury size and modulation of the inflammatory environment through the secretion of IGF-1, VEGF, EGF, BNDF and bFGF.
Leu et al. (2010)	• ASCs based therapies enhanced angiogenic and neurogenic processes in IS models.
Xin et al. (2013a)	• Systemic administration of exosomal fraction of the secretome impacted neurovascular plasticity.

IGF, Insulin Growth Factor; VEGF, Vascular Endothelial Growth Factor; EGF, Epidermal Growth Factor; BDNF, Brain Derived Neurotrophic Factor; bFGF, basic Fibroblast Growth Factor; ASCs, Adipose Tissue Stem Cells.

feature of stem cells that renders them as attractive tools for cancer therapy is their intrinsic capacity to migrate towards pathologic tissues, including malignant tumors. Indeed, this selective cancer-tropism has been shown for various stem cell types, including embryonic, hematopoietic, mesenchymal, neural, endothelial, and experimentally-induced stem cells (e.g., inducible pluripotent stem cells, iPSCs; Stuckey and Shah, 2014). Whether this innate tropism of normal stem cells is associated with cancer promotion or suppression functions is still controversial and a matter of debate, particularly in the case of MSCs, as reported by contradicting findings in many studies (Klopp et al., 2011). Nonetheless, it is widely consensual that the rational engineering of stem cells to express or deliver anticancer therapeutic agents, while taking advantage of their innate tumor tropism and immunosuppressive properties, may be a promising strategy to target cancer.

Aboody et al. (2000) first showed that NSCs are able to migrate towards the major tumor site and track along with invading glioma cells that form small satellite tumor masses (Aboody et al., 2000). Importantly, this tumor-tropism by NSCs was also later observed towards brain metastasis derived from breast cancer (Joo et al., 2009) and melanoma (Aboody et al., 2006), highlighting the potential application of NSCs as therapeutic vehicles for primary and metastatic brain tumors. In this context, and because stem cells are relatively easy to be genetically modified, many studies have explored them as cargo delivery vehicles for therapeutic agents, including cytokines, pro-drug converting enzymes, oncolytic viruses, nanoparticles, and antibodies, as summarized below.

Cytokines

Many recent studies have explored NSCs as efficient delivery systems of soluble tumor necrosis factor-related apoptosis-inducing ligand (sTRAIL), a cytokine that promotes apoptosis by binding to death receptors commonly present in the cellular membrane of tumor cells. These engineered NSCs can track tumor cells and deliver sTRAIL to glioma cells *in vivo*, resulting in significant anti-tumor effects. Combinations of sTRAIL-secreting NSCs with anticancer drugs, including bortezomib (a proteasome inhibitor), PI-103 (a dual PI3K/mTOR inhibitor), and lanatoside C (a cardiac glycoside), resulted in synergistic therapeutic effects, emphasizing the potential clinical value of sensitizing glioma cells to TRAIL-induced NSC-mediated cell death (Hingtgen et al., 2010; Bagci-Onder et al., 2011; Balyasnikova et al., 2011; Teng et al., 2014). Importantly, studies with MSCs engineered to deliver sTRAIL showed equally promising results, as these cells efficiently tracked and successfully induced a caspase-dependent cell death in glioma cells, resulting in increased survival of glioma mice models (Shah et al., 2004; Menon et al., 2009; Sasportas et al., 2009; Choi et al., 2011).

NSCs have also been genetically modified to express and secrete IL-12, a cytokine that does not act directly in tumor cells, but is involved in the enhancement of T-cell-mediated antitumor immune responses. Using intracranial glioma mice models, Ehtesham et al. showed that IL-12-secreting NSCs injected directly in the tumor significantly prolong the survival of mice (Ehtesham et al., 2002). Similarly, MSCs genetically engineered to express a modified IL-12 also prolonged the survival of glioma mice models when injected intratumorally (Ryu et al., 2011). Similar approaches were used to engineer NSCs, MSCs, and bone marrow-derived stem cells to produce pro-inflammatory cytokines, including IL-4, IL-7, IL-23, and IFN-β, which were shown to increase the infiltration of anti-tumor T-cells and natural killer (NK)-cells in glioma murine models (Benedetti et al., 2000; Nakamizo et al., 2005; Yuan et al., 2006; Gunnarsson et al., 2010). These studies provide important proof-of-concept on the potential of modulating immune mediators with different types of stem cells in order to achieve increased therapeutic responses.

Enzymes/pro-drugs

Another novel approach involves the modification of stem cells to express enzymes that convert inactive pro-drugs into toxic compounds, in order to increase tumor tissue selectivity. One of the most popular pro-drug/enzyme therapeutic systems is the herpes simplex virus type 1 thymidine kinase (HSV-tk) in combination with the pro-drug ganciclovir (GCV), based on the HSV-tk-mediated phosphorylation of inert GCV into a cytotoxic product that kills HSV-tk-positive cells and neighboring cells (via the so-called bystander effect). Taking advantage of the tumor-tropism of stem cells, many recent studies have explored the incorporation of HSV-tk into NSCs, MSCs, and bone marrow-derived progenitor cells as therapeutic

strategies for glioma, showing promising results (Li et al., 2005; Uhl et al., 2005; Miletic et al., 2007; Uchibori et al., 2009; Matuskova et al., 2010). Other enzyme/pro-drug systems that have been explored as anti-cancer therapeutic tools for stem cells include the cytosine deaminase (CD), which converts inactive 5-fluorocytosine (5-FC) into the cytotoxic 5-fluorouracil (5-FU), and the rabbit carboxylesterase enzyme (rCE), which converts the pro-drug CTP-11 (irinotecan) into the anticancer topoisomerase I inhibitor SN-38 (7-ethyl-10-hydroxycamptothecin). These approaches have been tested with promising therapeutic results in stem cells of different origin (NSCs and MSCs) and distinct glioma models (including rat and mice models), either alone or in combination with other anticancer drugs (Aboody et al., 2000, 2006; Lim et al., 2011; Yin et al., 2011; Choi et al., 2012; Fei et al., 2012; Kim et al., 2012; Kosaka et al., 2012; Ryu et al., 2012; Zhao et al., 2012), emphasizing the potential of these enzyme/pro-drug systems as stem cell-mediated anti-tumor therapies.

Oncolytic viruses

The use of oncolytic viruses as therapeutic agents has been extensively studied for cancer, taking advantage of their capacity to infect, replicate within, and ultimately kill cancer cells. Despite many promising pre-clinical studies, including in gliomas (Wollmann et al., 2012), the clinical application of oncolytic viruses presents critical obstacles, including sub-optimal distribution throughout the major tumor cores and particularly to invading cancer cells, low infection rates, and host anti-viral immune responses (Yamamoto and Curiel, 2010). Critically, these shortcomings can be largely surpassed by the incorporation of oncolytic viruses within tumor-trophic stem cells. Indeed, recent work has been performed in NSCs, MSCs and ASCs that were used as oncolytic viral carriers to treat *in vivo* models of glioma, showing that these cells retain tumor-tropism, permit continued viral replication for several days, and cause glioma cell death *in vivo* more efficiently than viral delivery alone (Herrlinger et al., 2000; Sonabend et al., 2008; Tyler et al., 2009; Yong et al., 2009; Josiah et al., 2010; Ahmed et al., 2011; Thaci et al., 2012).

Nanoparticles and antibodies

In the last 4 years, some studies also started to explore MSCs as delivery vehicles of drug-loaded nanoparticles and antibodies to target glioma. This strategy aims to improve the capacity of these agents to cross the blood-brain barrier, while minimizing toxic side effects caused by intravenous administrations. The results obtained to date indicate that these cells can successfully deliver nanoparticles (e.g., lipid nanocapsules loaded with ferrociphenol and membrane-anchored silica nanorattle–doxorubicin) and antibodies (e.g., cell surface-bound single-chain anti-EGFRvIII) to glioma cells *in vivo*, resulting in increased anti-tumor responses (Balyasnikova et al., 2010; Roger et al., 2010, 2012; Li et al., 2011).

In conclusion, a wide variety of stem cells hold great promise as novel therapeutic tools for the treatment of therapy-insensitive malignant brain gliomas. Some hallmarks of these cells that are critical for this purpose include their high tumor-trophic migration and tracking capacity, peculiar immunosuppressive properties, and easy genetic manipulation for cargo delivery. Nonetheless, inherently to its innovative nature and similarly to other experimental glioma therapies attempted in the past, several issues will certainly need to be addressed in order to translate these promising pre-clinical findings into clinically-relevant therapies for patients. Some of the obstacles that may be envisaged include the proper selection of the best stem cell type/origin, choice of the most appropriate cargo for each tumor type or personalized to specific patients, optimization of administration routes and dosing, evaluation of the long-term cell fate of engrafted stem cells (which may conceptually also form tumors or differentiate aberrantly in the target tissue/organ), and development of real-time imaging systems for therapeutic stem cells *in vivo*. The recent literature on this topic is very promising, but a concerted and integrated effort in this field will still be crucial to definitely pave the way to better treat patients, most likely integrating the rational use of particular stem cell-based approaches to act synergistically in concert with surgery, radiation and chemotherapy.

Conclusion

It is now evident that cells derived from the osteogenic and neurogenic niches present important interactions that may impact the future development of CNS related therapies. As discussed in the present review there is robust evidence showing that MSCs and their secretome are able to modulate the action of neurogenic niches and neural progenitors. Their usage was shown to promote the functional recovery of animal models of PD and stroke, as well as the application of novel paradigms for glioblastoma therapies. Nevertheless, it is still a largely unexplored field, with many questions yet to be addressed. For instance, are the traditional growth factors the main mediators of the actions promoted by the MSCs secretome; or, instead, do MSCs-derived unknown neuroregulatory molecules modulate such actions? Can we modulate the tropism that these cells display towards gliobastomas? So far, most of the studies focused on the action of MSCs towards the neurogenic niches, namely NSCs. However, few address if and how the neurogenic niches, and within them NSCs, modulate the action of MSCs. In fact a bidirectional communication between both cell types is most likely to occur. The answer to this and other questions will be important to further define this field in the future, and its impact in future CNS regenerative strategies.

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REVIEW
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Are neural crest stem cells the missing link between hematopoietic and neurogenic niches?

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Hematopoietic niches are defined as cellular and molecular microenvironments that regulate hematopoietic stem cell (HSC) function together with stem cell autonomous mechanisms. Many different cell types have been characterized as contributors to the formation of HSC niches, such as osteoblasts, endothelial cells, Schwann cells, and mesenchymal progenitors. These mesenchymal progenitors have themselves been classified as CXC chemokine ligand (CXCL) 12-abundant reticular (CAR) cells, stem cell factor expressing cells, or nestin-positive mesenchymal stem cells (MSCs), which have been recently identified as neural crest-derived cells (NCSCs). Together, these cells are spatially associated with HSCs and believed to provide appropriate microenvironments for HSC self-renewal, differentiation, mobilization and hibernation both by cell-cell contact and soluble factors. Interestingly, it appears that regulatory pathways governing the hematopoietic niche homeostasis are operating in the neurogenic niche as well. Therefore, this review paper aims to compare both the regulation of hematopoietic and neurogenic niches, in order to highlight the role of NCSCs and nervous system components in the development and the regulation of the hematopoietic system.

Keywords: hematopoietic stem cell, niche, neural crest stem cell, neural stem cell, signaling pathways

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Introduction: Adult Stem Cells Niches in the Adult Bone Marrow and Brain

Stem cells are characterized by their continuous self-renewal ability and pluri- or multipotentiality, and could consequently give rise to a wide panel of cell types. Non-germinal stem cells are classified into different categories. Embryonic stem cells (ES) are found in the inner cell mass of the blastocyst and are pluripotent stem cells that generate any mature cell of each of the three germ layers. Somatic stem cells are tissue-specific and more restricted than ES cells in terms of fate choice and of differentiation capabilities. They can be isolated from various fetal and adult tissues, and therefore constitute an attractive supply of material for cell therapy.

Abbreviations: BM, bone marrow; BMP, bone morphogenic protein; HSC, hematopoietic stem cell; MSC, mesenchymal stem cell; NCC, neural crest cell; NCSC, neural crest stem cell; NSC, neural stem cell; SVZ, subventricular zone.

Stem cell niches were deeply analyzed over these last years in order to better understand and control stem cell proliferation and differentiation. Indeed, the concept of niche refers to a microenvironment harboring stem cells, which regulates both their self-renewal property and cell fate choice. During embryonic development, various factors inside the niche act on stem cells and modify gene expression to induce their proliferation or differentiation, in order to favor the development of the fetus.

Within the adult human body, the main role of those niches is the maintenance of stem cell quiescence. Mammalian adult stem cell niches have been described in many tissues including the testis, the hematopoietic tissue, the skin, the intestine, or the brain. Several important factors regulate stem cell characteristics within the niche, such as adhesion molecules that mediate important cell-cell interactions between stem cells and supportive cells, neighboring differentiated cells or matrix components. In some cases of tissue injury, the surrounding environment acts on the niche and actively recruits stem cells to either self-renew or differentiate, to generate new cells and tissues. In the following paragraphs, we will more precisely focus on hematopoietic stem cell and neural stem cell niches (**Figure 1**).

The concept of hematopoietic niche was first introduced in 1978, when Schofield and collaborators observed that surrounding bone marrow stromal cells strongly supported hematopoietic stem cells (HSCs) maintenance and activity in an *in vitro* co-culture system, while spleen cells were less efficient in insuring HSC regulation (Schofield, 1978). According to Schofield and others, the HSC niche can be defined as an heterogeneous microenvironment inside the trabecular bone cavity, which is composed of specialized cell populations that play essential(s) role(s) in regulating the self-renewal and differentiation of HSC through both surface-bound factors and soluble signals, together with mature progeny released into the vascular system (Uccelli et al., 2008; Renstrom et al., 2010). Two functional subdivisions of HSC niches are described in the adult bone marrow (BM): (1) the endosteal niche is composed inter alia by osteoblasts lining the endosteum (Nilsson et al., 2001; Calvi et al., 2003; Zhang et al., 2003) and regulates HSC's quiescence by maintaining them in G0/G1 phase (Emerson, 2007); whereas (2) vascular niches host HSCs in close relationships with vascular endothelium of marrow sinuses and mostly embraces HSC proliferation, differentiation, and recruitment (Kiel et al., 2005; Kiel and Morrison, 2008). Maintenance of the stem cell pool and formation of differentiated progenitors are therefore harmonized in order to achieve a steady-state hematopoiesis.

Even if the cellular composition of HSC niches still remains elusive at some points, mesenchymal stem cells (MSCs) of the BM stroma are well-known cellular components of the HSC niche which regulate hematopoietic processes through the secretion of many growth factors and cytokines (see below) (Anthony and Link, 2014). In addition, *in vivo* reconstitution of the hematopoietic niche may be achieved upon transplantation of MSCs or of a subpopulation of osteoprogenitors, which tightly interact with sinusoids and secrete growth factors (Caplan, 1991; Muguruma et al., 2006; Sacchetti et al., 2007). Many studies also demonstrated the implication of perivascular cells (Crisan et al., 2008; Ramasamy et al., 2014) in the regulation of hematopoiesis. Interestingly, Méndez-Ferrer and collaborators recently shown that nestin$^+$ MSCs are essential components of the endosteal niche and are required for the proper regulation of hematopoietic processes (see below) (Mendez-Ferrer et al., 2010; Isern et al., 2014). More recently, they demonstrated that those nestin$^+$ MSCs were neural crest-derived stem cells (Isern et al., 2014), which are known to persist in the adult bone marrow and in various other adult tissues such as the skin or the dental pulp (Nagoshi et al., 2008; Achilleos and Trainor, 2012). Together with the identification of non-myelinating Schwann cells inside the bone marrow (Yamazaki et al., 2011), those findings highlight the contribution of nervous system elements (and more particularly the neural crest) to the formation and maintenance of the hematopoietic system.

As first demonstrated in the late 90's (Eriksson et al., 1998; Doetsch et al., 1999; Gage, 2000), the adult nervous system also shelters specific microenvironments that both support the maintenance of neural stem cells (NSCs) alongside with the generation of newborn cells, mostly neurons in adulthood (Zhao et al., 2008). Neurogenic sites are located within (1) the subventricular zone (SVZ) along the wall of lateral ventricles, where NSCs give rise to neurons migrating in the olfactory bulb and the striatum (Ernst et al., 2014), and (2) in the hippocampal subgranular zone, where NSC-derived neurons integrate the *dentatus gyrus*. NSC maintenance and neurogenesis are well-regulated by numerous signals provided by the local blood vessels network with highly specialized properties (Shen et al., 2004; Tavazoie et al., 2008), the cerebrospinal fluid that circulates along the ventricles (Silva-Vargas et al., 2013), and by the surrounding cells (Tavazoie et al., 2008).

Although NSC niches are central nervous system (CNS) structures that are not supposed to hold neural crest-derived cells, it appears that many similarities and connections between HSC and NSC niches could be revealed by the recent literature and presented in **Figure 1**. As mentioned before, this review aims to compare what is known about the mechanisms that regulate both hematopoietic and neurogenic events, with a focus on the potential roles of neuroectodermal-derived cells (NCSC in hematopoiesis and NSC in neuropoiesis) in the orchestration and the regulation of the adult stem cell niches.

Cellular and Molecular Regulation of Hematopoietic and Neurogenic Processes

As mentioned before, adult stem cell niches have been described in many different tissues. Despite significant anatomical differences, those tissues share many common features concerning the extracellular mechanisms by which the stem cell population is regulated within the niche (Zapata et al., 2012). We therefore decided to compare hematopoietic and neural stem cell niches, and to have a look on their regulation pathways (**Figure 2**).

CXCL12: The Most Important Cytokine Signalization for Stem Cell Homing and Maintenance

CXCL12 (also called stromal-derived factor 1 or SDF-1) is a member of the chemoattractive cytokine family (chemokines),

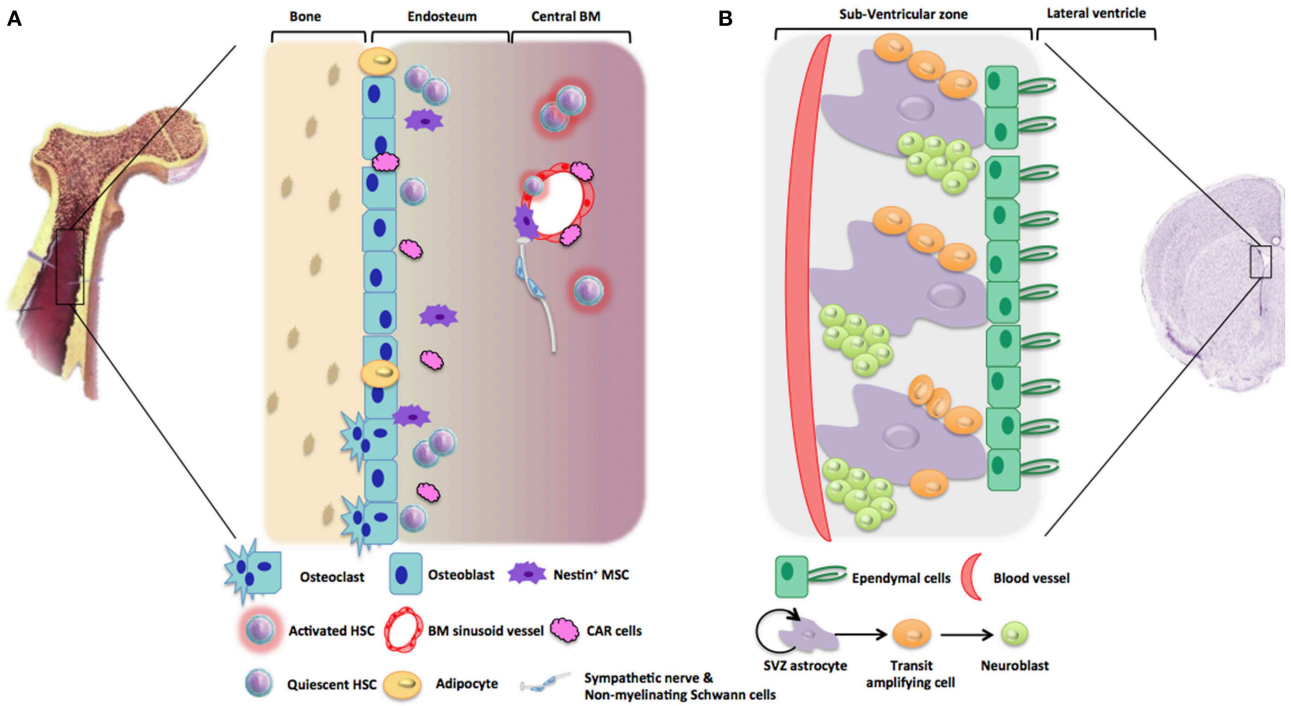

FIGURE 1 | Representative architecture of hematopoietic and neurogenic niches. (A) Hematopoietic niches are microenvironments into bone marrow stroma which support HSC quiescence and self-renewal. Endosteal niche is mainly composed of osteoblasts, osteoclasts, adipocytes, CAR cells, stromal cells, nestin$^+$ MSC, altogether maintaining HSC in a quiescent state. In comparison, vascular niche located near to BM sinusoid vessels is composed of perivascular (nestin$^+$) stromal cells and CAR cells favoring HSC activation and recruitment. Non-myelinating Schwann cells were also described to be involved in HSC maintenance. **(B)** The subventricular zone (SVZ) is one of the neurogenic niches in the adult brain. This neurogenic niche is in contact with ependymal cells that line the cerebrospinal fluid (CSF) circulating in the lateral ventricles. Neural stem cells (NSC) are localized in contact with this ependymal cell layer and with blood vessels. Type B cells or SVZ astrocytes proliferates thanks to asymmetric division giving rise to type C cells, or transit amplifying cells, which will further differentiate into type A cells or neuroblasts. During adult neurogenesis, these neuroblasts will proliferate and migrate toward the olfactory bulb or the striatum.

and is essential for the proper proceedings of hematopoiesis, general ontogeny, cardiovascular formation, and neurogenesis. Indeed, it has been observed that CXCR4-deficient mice (lacking the receptor for CXCL12) die around birth and present important defects in hematopoietic and nervous system, such as a reduced myelopoiesis and B-lymphopoiesis, and impaired neuronal migration in the cerebellum (Ma et al., 1998).

During bone marrow ontogeny, colonization of the bone marrow (BM) involves recruitment and engraftment of circulating myeloid cells and HSCs originating from the fetal liver, which will then interact with BM endothelium in order to migrate toward endosteal or vascular niches (Ara et al., 2003). This capture step is mainly driven by the secretion of CXCL12 by CXCL12-abundant reticular (CAR) cells (a subset of perivascular stromal cells), which acts on CXCR4 receptor at the surface of HSCs (Sugiyama et al., 2006). More recently, Isern and collaborators demonstrated that the capture step was also regulated by the presence of post-migratory NCSCs located in the BM niche. Those NCSCs secrete CXCL12 and attract HSCs that colonize the BM tissue in newborn mice (Isern et al., 2014).

Throughout adult life, maintenance of HSC quiescence, survival and self-renewal in the adult BM niche also relies on CXCL12/CXCR4 signalization by nestin$^+$ mesenchymal stem cells, CAR cells, osteoblasts, and endothelial cells, which differentially regulate the niche homeostasis (Greenbaum et al., 2013). Noteworthy, CXCL12-CXCR4 axis seems to be conserved during the evolution. Indeed, it was recently reported that zebrafish HSC homing in BM perivascular niche is dependent of CXCL12-expressing fibroblastoïd stromal cells, homologous of the CAR cells (Tamplin et al., 2015). Importantly, although CXCR4 is the most described receptor for CXCL12, it appears that another receptor (namely CXCR7) also has important role in HSC regulation and dysfunction (Melo Rde et al., 2014; Torossian et al., 2014).

In comparison, in the developing brain, CXCL12-CXCR4 axis regulates the migration of neuronal precursors in the cerebellum (Vilz et al., 2005), the dentate gyrus (Bagri et al., 2002; Kolodziej et al., 2008), the cerebral cortex (Stumm et al., 2003), the dorsal root ganglia (Belmadani et al., 2005) and some nuclei in the brainstem and hypothalamus (Schwarting et al., 2006). The other receptor for CXCL12, CXCR7 (Sanchez-Martin et al., 2013), also seems to be involved in CXCL12 signalization during brain ontogeny and homeostasis (Schonemeier et al., 2008). In the adult brain, CXCL12 and its receptors are expressed by a

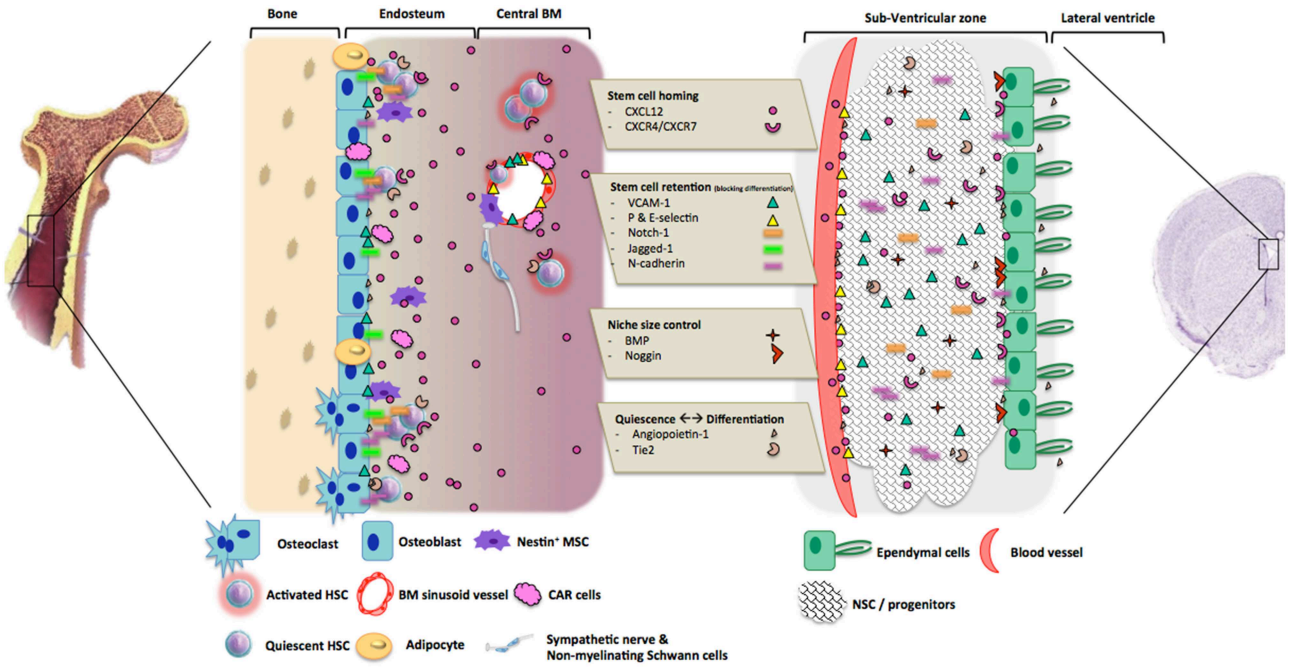

FIGURE 2 | Molecular processes involved in hematopoietic and neurogenic niches regulation. The molecules that are involved in the regulation of hematopoiesis and neurogenesis could be divided into three main groups, according to their roles in niches. 1. Stem cell homing: the couple CXCL12/CRCR4-7 are the most important component as they allow HSC or NSC homing and stemness into the niche. 2. Stem cell retention. After stem cells recruitment and homing, adhesion molecules and their ligands are involved in stem cells retention by blocking their differentiation and migration. 3. Control of niche size: BMP signaling pathway regulates niches size through the promotion of stem cells differentiation and or mobilization. 4. Control of quiescence and differentiation in HSC and NCSC niches: Angiopoietin-1 and its receptor Tie2 play different roles within the two niches from HSC quiescence maintenance to NSC proliferation and differentiation. Other actors could be involved, like sympathetic neurons regulating HSC attraction and mobilization into the blood flow.

lot of different neuronal populations located in the cortex, the mesencephalon or the hypothalamus (Banisadr et al., 2003). This chemokine is also secreted by ependymal cells and endothelial cells of the SVZ (Kokovay et al., 2010; Goffart et al., 2015), which both form a vascular neurogenic niche and contribute to the maintenance of stemness/migration in the adult brain (Shen et al., 2008).

VCAM1 and N-cadherin: Homing and Balance between Stem Cell Retention and Migration

Homing of HSCs during development into BM also involves cell-cell interactions. Those are mediated by adhesion molecules expressed by BM sinusoidal endothelial cells and stromal cells (Simmons et al., 1992), such as vascular cell adhesion molecule 1 (VCAM1), P-selectin and E-selectin (which respectively attract circulating HSCs by acting on α4β1 integrin, CD162 and E-selectin ligands) (Frenette et al., 1998). The expression of VCAM1 on those cells is also responsible for the regulation of normal cell trafficking between the BM and the blood stream in adult individuals (Ulyanova et al., 2005). Of note, additional ligands of α4β1 integrin, namely osteopontin and fibronectin, are also involved in maintaining HSCs in a quiescent state (Jiang et al., 2000; Nilsson et al., 2001; Stier et al., 2005). Similarly, VCAM1 is expressed by neural precursors in the adult brain SVZ and largely contributes to the niche architecture and function. Indeed, it appears that VCAM1 maintains NSC in a stem cell state by inducing the formation of reactive oxygen species (Le Belle et al., 2011). Just as in the adult BM, VCAM1 acts as a sensor and modulates stem cell maintenance/migration in response to environmental signals (Kokovay et al., 2012).

In the developing bone marrow as well as in the adult hematopoietic tissue, homotypic N-cadherin-mediated cell interactions between spindle-shaped N-cadherin expressing osteoblasts (SNOs) and HSCs are critical for regulating stem cell engraftment and quiescence, in the endosteal niche (Zhang et al., 2003). However, since KO mice for N-cadherin do not develop further than mid-gestation (Radice et al., 1997), there is therefore no functional evidence for N-cadherin role in the bone marrow. Even though, other molecular pathways also contribute to stem cell retention (Kiel et al., 2007). As an example, angiopoietin-1-dependent regulation of N-cadherin increases HSC adhesion within the endosteal niche (Arai et al., 2004). During cerebral cortical development, N-cadherin-mediated interactions between precursors within the ventricular zone coordinate signaling pathways that regulate proliferation and differentiation. N-cadherin-dependent cell contact regulates β-catenin signaling though Akt activation, and precursors thus regulate their own differentiation, survival and migration (Zhang et al., 2010, 2013).

N-cadherin also mediates NSC anchorage to ependymal cells and quiescence within the SVZ, while suppression of N-cadherin function promotes NSC migration and differentiation (Yagita et al., 2009). This interaction is regulated by membrane-type 5 metalloproteinase (MT5-MMP), which dynamically modulate the proliferative status of NSCs through cleavage of N-cadherin adhesive contacts (Porlan et al., 2014). In pathological conditions, N-cadherin interactions could also be disrupted by ADAM10, which induces cytoskeletal rearrangements in NSC and migration from the SVZ toward demyelinated lesions (Klingener et al., 2014).

Angiopoietin-1: from Quiescence to Differentiation

Angiopoietin-1 is an endothelial growth factor that is critical for division, survival, and adhesion of endothelial cells, via its tyrosine kinase receptor Tie-2 (Suri et al., 1996). Within the endosteal niche, HSCs are maintained in a quiescent state thanks to the secretion of angiopoietin-1 by osteoblasts, acting on Tie-2 receptor at the surface of HSCs (Arai et al., 2004).

In the adult brain, perivascular astrocytes, endothelial cells, ependymal cells, and choroid plexus are sources of angiopoietin-1. On the other hand, Tie-2 is express by non-endothelial cells, especially in neurons and stem cells from human and mouse brain, but also in glia (and glioblastoma cells (Rosa et al., 2010). *In vitro* studies show that angiopoietin-1 has pro-neurogenic effect through Tie-2 activation, and promote neurite outgrowth and synaptogenesis in sensory neurons (Kosacka et al., 2005, 2006). Angiopoietin-1 stimulates adult SVZ-derived NSC proliferation *in vitro*, and also increases differentiation in functional neurons and axonogenesis (Rosa et al., 2010). Angiopoetin-2 (another member of angiopoietin growth factors) also acts on Tie2 receptor and promotes NSC differentiation into neuronal lineage, and regulates neural progenitor cell migration through MMPs activity (in a Tie2-independent manner) (Liu et al., 2009).

BMP Signaling Pathway: Controlling Niche Size and Stem Cell Differentiation

Bone morphogenic proteins (BMPs) are members of the transforming growth factor β family. Among them, BMP4 signaling regulates mesoderm cell commitment into HSC and differentiated myeloid cells during embryogenesis and hematopoietic tissue development (Chadwick et al., 2003; Durand et al., 2007) (reviewed in Sadlon et al., 2004). Moreover, BMP4 is expressed in osteoblasts, endothelial cells, and megakaryocytes (Goldman et al., 2009), and is involved in bone marrow niche homeostasis in adulthood by controlling HSC number and preserve niche size (by signaling through BMP receptor type IA) (Zhang et al., 2003). Interestingly, it appears that SMAD-dependent BMP signaling also regulates CXCL12 secretion in the BM niche, then influencing homing, engraftment, and mobilization of HSCs (Khurana et al., 2014).

In the developing brain, BMPs induce astroglial and neuronal differentiation of NSCs and precursors in the embryonic SVZ and developing cortex (Gross et al., 1996; Li et al., 1998), and inhibit neurogenesis (Shou et al., 1999).

BMPs are also expressed in the adult SVZ where they prevent neuroblast production from precursors by directing them into a glial lineage. However, neurogenic environment is maintained by ependymal cells secreting Noggin, which inhibits BMP signaling in the SVZ and stimulates neurogenesis (Lim et al., 2000).

Notch Signaling Pathway: Role in Expansion of Undifferentiated Stem Cells

Notch signaling plays fundamental role in embryogenesis by mediating cell proliferation, cell differentiation and cell fate decision (Artavanis-Tsakonas et al., 1999). A Notch-mediated crosstalk takes place in the BM niche, wherein Notch-1 is expressed by HSCs (and by other mature blood cell types) (Milner et al., 1994) and its ligand Jagged-1 is expressed by osteoblasts. The expression of Jagged-1 by the endosteal niche cells is stimulated by the parathyroid hormone (Calvi et al., 2003). Interestingly, Jagged-1 expression could also be identified in NCSC from adult mouse bone marrow, using a micro-array approach (GSE30419) (Wislet-Gendebien et al., 2012). Notch-1 signalization enhances stem cell renewal, but also favors lymphoid lineage and particularly T-cell differentiation (Bigas and Espinosa, 2012).

Similarly, in the adult SVZ, Notch signaling plays a role in the maintenance of stem cell population, and inactivation of the pathway depletes NSC pool and induces neuronal differentiation. More precisely, in human developmental neocortex, Notch signaling maintains a pool of progenitor cells called non-ventricular radial glia-like cells, which are able to differentiate into neurons (Hansen et al., 2010). This mechanism is regarded as a critical evolution step allowing the increase of neuron number in human telencephalon. Moreover, it was also reported that Notch actively cooperates with the pathway triggered by the EGF-receptor to balance the neural stem cells population with the neuronal precursor population in the adult SVZ (Aguirre et al., 2010).

Nervous System Regulates Stem Cells Homing and Exit from Their Niche

Both adult hematopoietic and neurogenic regions depend critically on nervous system signals. Indeed, sympathetic noradrenergic neurons regulate the attraction of HSCs to their niche, and their mobilization into the blood flow, in cooperation with G-CSF (Katayama et al., 2006). Furthermore, it appears that a denervation of autonomic nerves in the BM leads to a reduced number of non-myelinating Schwann cells (contributing to HSCs maintenance through TGFβ signaling) (Yamazaki et al., 2011). As already mentioned, these non-myelinating Schwann cells have close similarities with NCSCs.

Neuronal afferences contacting the adult SVZ are also known to regulate many parameters of the niche, according to the neurotransmitters that are secreted (reviewed in Young et al., 2011). Neurogenesis is therefore impaired in pathological conditions such as Parkinson's disease, when afferences from the striatum are lost (L'Episcopo et al., 2012).

Could Neural Crest Stem Cells from HSC Niches Explain the Similarities between Hematopoietic and Neurogenic Niche Signals?

During development, neural crest cells (NCCs) constitute a transient population of multipotent cells that arise at the border of the neural plate. After induction, NCCs delaminate, undergo epithelial-to-mesenchymal transition and migrate in discrete streams (cardiac, trunk, cranial, vagal NCCs) toward different tissues, finally giving rise to neurons and glia of the peripheral nervous system, melanocytes, craniofacial osteocytes, chondrocytes, etc. (Achilleos and Trainor, 2012; Mayor and Theveneau, 2013). Beside a well-determined transcriptional regulation (Anderson, 1994; Hong and Saint-Jeannet, 2005), numerous extracellular signals, growth factors, and adhesion molecules finely regulate different parts of this sequence.

CXCL12/CXCR4 Axis

The role of CXCL12/CXCR4 signalization axis in the migration of neural crest cells during development is well-defined. CXCL12/CXCR4 (and not CXCR7) chemoattractant signaling is required for the proper progression and migration of cardiac neural crest cells (NCCs) toward their appropriate locations in the developing heart (Escot et al., 2013) as well as for the correct development of craniofacial/orofacial cartilages that result from cranial NCCs migration (Olesnicky Killian et al., 2009; Rezzoug et al., 2011). This signaling axis is also required for the migration of sensory neurons and DRG formation (Belmadani et al., 2005). Overall, data of the literature underlines the importance of CXCL12/CXCR4 signaling during NCC migration. Still, it appears that NCCs rather respond to environmental secretion of CXCL12 instead of producing it themselves, as it is the case for adult bone marrow NCSCs (Isern et al., 2014).

Adhesion Molecules—VCAM-1 and N-cadherin

Migratory NCCs progress along defined pathways and cell adhesion molecules are required to allow NCC interactions with each other and with environing tissues (reviewed in McKeown et al., 2013). They express the α4β1 integrin enabling them to respond to a VCAM1 stimulus (Testaz et al., 1999). However, the same study showed that the NCCs migration cannot be triggered by only VCAM1/α4β1 integrin interaction, but also requires also a fibronectin stimulus. NCC emigration from the neural tube is also mediated by N-cadherin, which is highly expressed in premigratory NCCs and then switched off in favor of weaker type II cadherins (Mayor and Theveneau, 2013). Indeed, its overexpression disrupts the proper cell migration pattern of NCCs (Nakagawa and Takeichi, 1998).

BMPs

Together with Wnt signaling, BMPs are important for the induction of neural crest in the earliest embryonic developmental steps (Raible, 2006). This important role of BMPs in NCC specification is also exemplified by the fact that NSCs put in culture and treated with BMP2 are induced to a neural crest fate and choroid plexus mesenchyme, after an epithelial-to-mesenchymal transition. The cells then differentiate into smooth muscle cells and peripheral nervous system glia (Sailer et al., 2005). Later during development, BMP2/4/7 derived from the wall of the dorsal aorta and surrounding mesenchyme induce NCCs to become precursors of sympathetic neurons and chromaffin cells, so-called sympatho-adrenal progenitors. They can be identified by their expression of distinct sets of transcription factors, most notably Phox2B, and components of the catecholaminergic synthetic machinery, as, e.g., tyrosine hydroxylase and dopamine ß-hydroxylase (reviewed in Unsicker et al., 2013). The inducing activity of BMPs in catecholaminergic neurons is also consolidated by the observation that BMPs are also able to stimulate NCC differentiation in enteric dopaminergic neurons (Chalazonitis and Kessler, 2012). This importance of BMPs in sympathetic and/or catecholaminergic neuron progenitor differentiation could also be involved in bone marrow regarding the implication of sympathetic innervation in the HSC niches (see above).

Notch

Notch was recently suspected to play a role in the NCCs differentiation. In self-renewing pre-migratory NCCs induced from human pluripotent stem cells, Noisa et al. observed that Notch increases the expression of neural-crest-specifier genes (*SLUG* or *SNAIL2*, *SOX10,* and *TWIST1*) (Noisa et al., 2014) Moreover, Notch is then a brake of NCCs migration and the inhibition of Notch signaling is followed by a neuronal differentiation of these cells. Using *in vitro* and *in vivo* models, Morisson et al. demonstrated that Notch inhibits NCCs neuronal differentiation and activates the glial fate, mainly the Schwann cell phenotype (Morrison et al., 2000a,b) but not the satellite cells, the teloglia of somatic motor nerve terminals or the enteric glia (reviewed in Kipanyula et al., 2014).

Conclusions

In light of this review, it appears that the relationship between hematopoietic and nervous systems, at least at the molecular level, has been under-estimated for many years. The main reason probably resides in the fact that the hematopoietic system has been well-described as a highly regenerating system for many years, while the nervous system is the ultimate example of a non-, or at least poorly-, regenerating system. However, the description of neurogenic niche regulation in the adult mammalian brain (including in humans) and the recent findings concerning several regulatory cell components of hematopoietic niches together shed the light on the obvious similarities concerning the molecular regulation pathways of the two systems. Moreover, increasing description of the nervous regulation of hematopoietic function, together with the putative importance of the relationship between SVZ vasculature and NSCs, seems to be the dawn of an interpenetration of both systems. Likewise, recent findings demonstrating that crayfish neurons are generated from the immune system is another example of this crisscross (Benton et al., 2014). We therefore suggest that in mammals, the interpenetration of both systems

relies, at least partly, on neural crest derivatives present in bone marrow. A better knowledge of the properties and the roles of these cells could shed a light on the hematopoietic niche regulation, but could also feed new hypotheses for exploring and understanding neural stem cell niches of the adult brain.

Finally, this review should deliver another important message concerning the common regulation modes of both systems and their possible common dysregulations in pathological conditions (e.g., in leukemias and gliomas). Indeed, in both systems, adult niches have been demonstrated to provide a sanctuary for subpopulations of leukemic, but also glioblastoma cells that escape chemotherapy- and/or-radiotherapy induced death. Indeed, xenografted glioblastoma cells were recently shown to migrate toward the SVZ upon stimulation by CXCL12, which is secreted by endothelial cells (Goffart et al., 2015). It appears that this NSC niche also constitutes a particular microenvironment that promote glioblastoma cell maintenance (Goffart et al., 2013). On the other hand, the hematopoietic niche is also a key environmental regulator of leukemia stem cell proliferation, survival, and migration (Tabe and Konopleva, 2014). In both cases, cancer stem cells seem to share important features with stem cells located in the niche and are likely to be equally influenced by these specific microenvironments. Further understanding about the molecular regulation of those niches and the possible roles of NCCs in this context is therefore of particular importance.

Author Contributions

CC: Work conception and design, data collection and analysis, manuscript writing. VN: Work conception and design, data collection and analysis, manuscript writing. AG: Revision for intellectual content, final approval of the version to be published. SW: Work conception and design, revision for intellectual content, final approval of the version to be published. BR: Work conception and design, revision for intellectual content, final approval of the version to be published.

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Purinergic signaling: a common pathway for neural and mesenchymal stem cell maintenance and differentiation

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Extracellular ATP, related nucleotides and adenosine are among the earliest signaling molecules, operating in virtually all tissues and cells. Through their specific receptors, namely purinergic P1 for nucleosides and P2 for nucleotides, they are involved in a wide array of physiological effects ranging from neurotransmission and muscle contraction to endocrine secretion, vasodilation, immune response, and fertility. The purinergic system also participates in the proliferation and differentiation of stem cells from different niches. In particular, both mesenchymal stem cells (MSCs) and neural stem cells are endowed with several purinergic receptors and ecto-nucleotide metabolizing enzymes, and release extracellular purines that mediate autocrine and paracrine growth/proliferation, pro- or anti-apoptotic processes, differentiation-promoting effects and immunomodulatory actions. Here, we discuss the often opposing roles played by ATP and adenosine in adult neurogenesis in both physiological and pathological conditions, as well as in adipogenic and osteogenic MSC differentiation. We also focus on how purinergic ligands produced and released by transplanted stem cells can be regarded as ideal candidates to mediate the crosstalk with resident stem cell niches, promoting cell growth and survival, regulating inflammation and, therefore, contributing to local tissue homeostasis and repair.

Keywords: purinergic receptors, ATP, adenosine, mesenchymal stem cells, neural stem cells

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Purinergic Ligands are Ancient and Widespread Mediators of Cell-to-Cell Communication

It is now widely accepted that in adult organisms stem cells contribute to tissue homeostasis and repair through paracrine mechanisms, along with a mere integration into existing tissue architecture (Wang et al., 2014). Trophic factors combined with immunomodulatory molecules often represent the main mechanism responsible for the functional improvements exerted by transplanted stem cells (Uccelli et al., 2008; Leatherman, 2013). Released nucleotides and nucleosides behave as trophic, differentiating, and immunomodulatory molecules in many physiological and pathological events, through autocrine and paracrine mechanisms (Glaser et al., 2012). Phylogenetically, purinergic ligands are considered ancient molecules involved in cell-to-cell communication, and their receptors are expressed by almost every cell type, even in very primitive organisms such as prokaryotes, protozoa, and early plants

(Burnstock and Verkhratsky, 2010). Purinergic receptors are also among the first neurotransmitter receptors to be expressed during very early stages of ontogenetic development (Burnstock and Ulrich, 2011). This conserved and widespread use of purinergic ligands for intercellular communication is possibly due to the fact that nucleotides (and ATP in particular) are fundamental constituents of cells, being the most widely used high energy carrier molecules, and because they are the building blocks of nucleic acids. Cells therefore usually contain millimolar concentrations of intracellular ATP that can be discharged into the extracellular space by vesicular exocytosis, concentrative, and equilibrative transporters, connexin/pannexin hemichannels and uncontrolled leakage from injured cells (Lohman et al., 2012).

Once released into the extracellular environment, purinergic ligands behave as signal mediators, activating different subtypes of purinergic receptors. There are four subtypes of adenosine P1 receptors (A1, A2A, A2B, and A3), seven subtypes of nucleotide P2X ligand-gated ion channel receptors (P2X1–7) and eight subtypes of nucleotide P2Y metabotropic receptors (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, and P2Y14). The P1 and P2Y subtypes are classical seven-transmembrane domain receptors, whose action is mediated through G-proteins and intracellular second messengers, including Ca^{2+}, cAMP, and $InsP_3$ (Burnstock, 2007).

The effects of ATP and adenosine are usually opposite and the resulting signal cascade activated by extracellular nucleotides and nucleosides in target cells is the combinatorial resultant of their extracellular metabolism, uptake and binding to specific receptors (Volonté and D'Ambrosi, 2009). Ecto-nucleotide metabolizing enzymes (in particular ecto-nucleoside triphosphate phosphohydrolases, and ecto-5′-nucleotidase) are powerful tools to control the effects mediated by extracellular purines, as they switch off the signal induced by ATP on P2 receptors, hydrolyzing it into adenosine, thereby activating P1 receptors.

Because of their widespread presence and the broad array of functions they can mediate, it is not surprising that purinergic receptors are involved in many aspects of stem cell physiology: mesenchymal stem cells (MSCs) and neuronal progenitor cells (NPCs) release and respond to purinergic ligands with altered proliferation, migration, differentiation and apoptosis, and by regulating immune responses associated with their mobilization (Burnstock and Ulrich, 2011). In this review we will analyze how purinergic signaling behaves as a common paracrine pathway that activates MSCs and neural stem cells (NSCs) in both physiological and pathological conditions.

Dual Role of the Purinergic System in NSCs in Physiological and Pathological Conditions

Extracellular Purines Modulate Adult Neurogenesis

Neural progenitor cells in adult brain express different purinergic receptors. Indeed, mRNAs for P2X4 and P2X7 subtypes, all P2Y receptors except P2Y4 and P2Y11, and all P1 receptors, but A3, have been found in subventricular zone (SVZ)-derived primary neurospheres (Stafford et al., 2007; **Table 1**). Moreover, neural progenitor cells of both SVZ and subgranular zone neurogenic niches highly express the nucleotide-metabolizing enzymes ectonucleoside triphosphate diphosphohydrolase (NTPDase) 2 and the tissue-non-specific alkaline phosphatase (TNAP; Langer et al., 2007). Extracellular nucleotides generated by these enzymes in the SVZ produce a rapid and transient increase in intracellular calcium mainly through the activation of the metabotropic P2Y1 receptor (Mishra et al., 2006). The role of P2Y1 in modulating neurogenesis changes depending on the physiological conditions and the concomitant presence of EGF and FGF. In fact, specific stimulation of this receptor in NPCs increases cell proliferation and migration (Grimm et al., 2010), but only when the growth factor concentration is low or absent (Mishra et al., 2006; Boccazzi et al., 2014; **Table 1**; **Figure 1A**). Conversely, when the growth factor concentration is higher, activation of P2Y1 has an antiproliferative effect (Stafford et al., 2007; **Table 1**). It was recently demonstrated that infusion of ATP in rat SVZ selectively increases the proliferation of type C cells but not of type B or A (Suyama et al., 2012). This effect is counteracted by the selective P2Y1 antagonist 20-deoxy-N6-methyladenosine-30,50-bisphosphate (MRS2179) suggesting a specific role of the P2Y1 receptor in modulating the activity of transit amplifying cells. In line with this, an additional indication of P2Y1 receptor functioning comes from evidence that ATP secreted by astrocytes, even at basal levels, promotes the proliferation of neural progenitor cells through activation of the P2Y1 subunit (Cao et al., 2013; **Figure 1A**).

The effect of P2Y1 in stimulating the proliferation of progenitor cells and neurogenesis can be counterbalanced by activation of the P2X7 receptor (**Figure 1A**). This receptor subtype can regulate the homeostasis of the neurogenic niche, limiting excessive neuro- and glio-genesis by inhibiting proliferation and stimulating NPC differentiation (Tsao et al., 2013) and activating apoptotic mechanisms (Delarasse et al., 2009; **Table 1**). The P2X7 receptor expressed on neuroblasts can also contribute to the clearance of apoptotic cells by activating innate phagocytosis during early stages of neurogenesis (Lovelace et al., 2015; **Table 1**).

Extracellular Purines Affect NSC Response in Pathological Conditions

Massive release of extracellular ATP is one of the hallmarks of neurodegeneration. After a pathological event in the brain, such as ischemia or Parkinson's disease all CNS cell types activate different purinergic receptors. P2X7, which is expressed mainly in microglia, astrocytes, and neurons, is the principal agent responsible for purinergic-induced excitotoxic cell death (Sperlagh et al., 2006). Activation of P2X7 in pathological conditions in neurons and astrocytes induces the formation of large pores which, together with pannexin channels, allow the passage of cations, the leakage of metabolites of up to 900 Da and further release of ATP. During an insult extracellular ATP can achieve millimolar concentrations in the extracellular space, determining sustained activation of purinergic receptors and an

TABLE 1 | Presence and function of purinergic P1 and P2 receptors in neural precursor cells and mesenchymal stem cells.

	Neural precursor cells (NPCs)		Mesenchymal stem cells (MSCs)	
P1/P2	**Presence**	**Effect**	**Presence**	**Effect**
A1	+	n.d.	+	Lipogenic activity Gharibi et al. (2011)
A2A	+	n.d.	+	Maintainace of osteoblastic differentiation; ↑ adipogenesis Gharibi et al. (2011)
A2B	+	n.d.	+	↑ Osteogenesis Ham and Evans (2012)
A3	n.d.	n.d.	+	n.d.
P2X1	n.d.	n.d.	+	n.d.
P2X2	n.d.	n.d.	n.d.	n.d.
P2X3	n.d.	n.d.	+	n.d.
P2X4	+	n.d.	+	n.d.
P2X5	n.d.	n.d.	+	↑ Osteogenesis Zippel et al. (2012)
P2X6	+	↓ Migration after ischemia Vergni et al. (2009)	+	↓ Osteogenesis Zippel et al. (2012)
P2X7	+	↓ Proliferation; ↑ neuronal differentiation Tsao et al. (2013) ↑ Apoptosis Delarasse et al. (2009), Messemer et al. (2013) ↓ Migration after ischemia Vergni et al. (2009) ↑ Innate phagocytosis Lovelace et al. (2015)	+	↑ Osteogenesis and mineralization Sun et al. (2013), Noronha-Matos et al. (2014)
P2Y1	+	↑ Proliferation Mishra et al. (2006), Boccazzi et al. (2014) ↑ Migration Grimm et al. (2010) ↓ Proliferation in the presence of high growth factor concentration Stafford et al. (2007)	+	↓ Proliferation Coppi et al. (2007) ↑ Adipogenesis Ciciarello et al. (2013)
P2Y2	+	↑ Proliferation Mishra et al. (2006) ↓ Migration after ischemia Vergni et al. (2009)	+	↓ Osteogenesis Zippel et al. (2012)
P2Y4	n.d.	n.d.	+	↑ Adipogenesis Zippel et al. (2012), Ciciarello et al. (2013)
P2Y6	+	n.d.	+	n.d.
P2Y11	n.d.	n.d.	+	↑ Adipogenesis Zippel et al. (2012) ↑ Proliferation, migration, cytochine release Fruscione et al. (2011)
P2Y12	+	n.d.	+	n.d.
P2Y13	+	n.d.	+	↑ Osteogenesis, ↓ adipogenesis Biver et al. (2013)
P2Y14	+	n.d.	+	n.d.

+, Presence; n.d., not detected; ↑, stimulation; ↓, inhibition. The presence of purinergic receptors in NSCs was established by Stafford et al. (2007), in MSCs by Ferrari et al. (2011) and Zippel et al. (2012).

increase in intracellular calcium in target cells. The imbalance of calcium homeostasis in microglia results in the release of different interleukins, triggering a neuroinflammatory reaction (Sperlagh et al., 2006). However, the role of neuroinflammation in modulating neurogenesis during a pathological event is still debated. Inflammatory cytokines have both a positive and a negative effect on neurogenesis (Borsini et al., 2015) and the activation of purinergic receptors on microglia and astrocytes plays a relevant role in modulating their release. For example, microglial P2X7 activated by its specific agonists ATP and benzoyl-ATP during neuronal stress modulates the expression of NOD-like receptor (NLR) P3 inflammasome (Franceschini

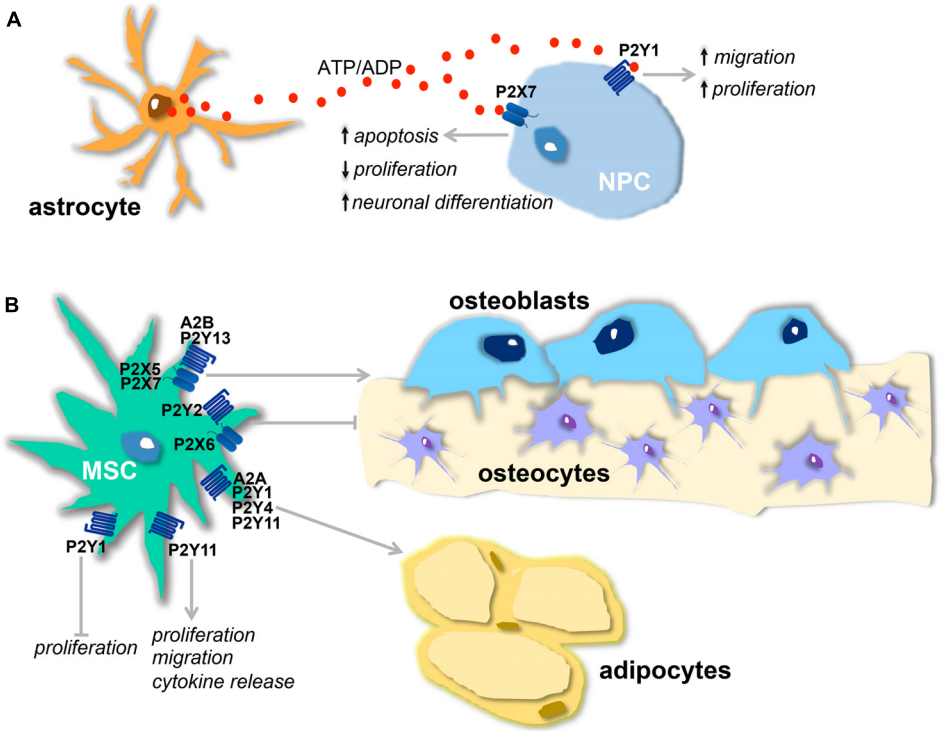

FIGURE 1 | Physiological effects of purinergic receptors in neural and mesenchymal stem cells (MSCs). (A) Proposed model of purinergic receptor action on neurogenesis: ATP, released from astrocytes, and ADP, resulting from ATP hydrolysis, stimulate, respectively, P2X7 and P2Y1 receptors present on neural stem cells (NPCs). The activation of P2Y1 receptor leads to increased proliferation and migration and this effect is counterbalanced by P2X7 activation that decreases proliferation, induces neuronal differentiation, and apoptosis. **(B)** Osteogenic and adipogenic actions of purinergic receptors present on MSCs : A2B, P2Y13, P2X5, and P2X7 receptors stimulate osteogenesis, while P2X6 and P2Y2 are inhibitory. A2A, P2Y1, P2Y4 and P2Y11 receptors are adipogenic. P2Y11 receptor also induces migration, cytokine release, and proliferation. Proliferation is inhibited by P2Y1 receptor.

et al., 2015), sustaining the release of proinflammatory cytokines which, in turn, may contribute to the inhibition of progenitor cell activity. Conversely, the increase in P2X4 expression in astrocytes contributes to CNS remodeling after trauma and further increases synaptogenesis (Franke and Illes, 2006). Brain ischemia is also characterized by the release of inflammatory cytokines. After an ischemic insult the SVZ is able to release factors that can protect against cortical damage (Cavaliere et al., 2006) and the purinergic system can inhibit this function. Indeed, ATP released after brain insult overstimulates P2 receptors expressed in SVZ progenitor cells (mainly P2X6, P2X7, P2Y1, and P2Y2; Stafford et al., 2007; Vergni et al., 2009), inhibiting the migration of neuroblasts to the damaged cortex (**Table 1**). This process is further enhanced by a locally decreased production of the chemoattractant SDf-1alpha and may also be reversed by blocking the activation of microglia (Vergni et al., 2009). In this case, purines, together with other death signals released by damaged cells, counterbalance the response of progenitor cells recruited after damage (Messemer et al., 2013; **Table 1**).

The general assumption is that, during an insult, ATP can act as a detrimental pro-inflammatory signal, whereas adenosine, mainly through A1 and A3 receptors, usually has opposite properties (Fiebich et al., 2014). It is well known that ATP released after brain injury can be hydrolized by NTPDase2, which is highly expressed in the neural progenitor cell membrane (Gampe et al., 2015), and generate adenosine that, together with the adenosine released directly during brain damage, also has a modulatory effect on neurogenesis (Ulrich et al., 2012).

Finally, an important role in the modulation of NSC function following a stressful event is also exerted by orphan G protein-coupled receptors, which can be activated by extracellular nucleotides. This is the case of GPR17, a novel P2Y receptor specifically activated by both uracil nucleotides (UDP, UDP-glucose, and UDP-galactose) and cysteinyl-leukotrienes (cysLTs; Blasius et al., 1998; Ciana et al., 2006). GPR17 is also expressed in neural progenitor cells, mainly oligodendrocyte precursor cells, and acts as a regulatory factor in mediating oligodendrocyte response and neuronal death after brain ischemia (Lecca et al., 2008).

Purinergic Signaling in MSCs

Mesenchymal stem cells are self-renewing multipotent stem cells with the capacity to differentiate into chondrocytes, osteoblasts, or adipocytes. Numerous studies have shown that many molecules, inorganic compounds, and mechanical agents contribute to their commitment in the different lineages and

it is now clear that there is an inverse relationship between their differentiation into osteoblatsts and into adipocytes. This balance is regulated by intersecting signaling pathways that converge on the regulation of two main transcription factors: peroxisome proliferator-activated receptor-γ (PPARγ) and Runt-related transcription factor 2 (Runx2), which are generally regarded as the master regulators of adipogenesis and osteogenesis, respectively (James, 2013).

Purinergic ligands have been widely described as early factors determining MSC fate (Glaser et al., 2012; Scarfì, 2014) but, while the role of the P1 receptors in MSC physiology is fairly clearly defined, the function of P2 receptors is more controversial, possibly because most of the 15 P2 receptor subtypes have been identified on MSCs (Zippel et al., 2012), it is often difficult to separate the effects of ATP from those of adenosine, and their function seems also to be influenced by the source of origin of the cells. To simplify, ATP can be considered both adipogenic and osteogenic, while its degradation product, adenosine, switches off adipogenic differentiation and has a prevalently osteogenic action (Gharibi et al., 2012; Ciciarello et al., 2013).

P1 Receptors on MSCs are Mostly Osteogenic and Immunomodulatory

Mesenchymal stem cells release adenosine and possess all P1 receptors (Evans et al., 2006), with A2B as the predominant subtype in undifferentiated cells and during osteoblastogenesis (Gharibi et al., 2011). Not only is adenosine released but most of it derives from the hydrolysis of ATP by ectonucleoside triphosphate diphosphohydrolase 1 (CD39) and ecto-5′-nucleotidase (CD73) activities that are abundantly present in the plasma membrane of MSCs (Sattler et al., 2011). Adenosine exerts an osteogenic action (Ham and Evans, 2012) mainly via the A2B receptor (**Table 1**; **Figure 1B**), its effects being canceled on pharmacological inhibition of this receptor subtype (He et al., 2013), and since overexpression of A2B receptors induces the synthesis of osteoblast-related genes (Runx2 and alkaline phosphatase; Gharibi et al., 2011). Consistently with these *in vitro* results, the knockout of CD73 in mice decreases osteoblast differentiation, resulting in osteopenia (Takedachi et al., 2012); A2B-deficient mice show impaired osteogenic differentiation, a mild osteopenic phenotype and impaired fracture physiology (Carroll et al., 2012); finally, loss of equilibrative nucleoside transporter 1 (ENT1) in mice, with consequent inhibition of adenosine reuptake, leads to ectopic calcification of spinal tissues (Warraich et al., 2013). Adenosine formation and activation of A2B receptors has also been strongly implicated in osteogenic differentiation induced by biomaterials containing calcium phosphate moieties (Shih et al., 2014). The A2A subunit has also been implicated in osteogenesis, being involved mainly in the maintenance of osteoblastic differentiation (**Table 1**) and this P1 subunit, together with the A1 receptor subtype, is also found upregulated during adipogenesis, influencing, respectively, differentiation (through upregulation of PPARγ; **Figure 1**) and lipogenic activity (Gharibi et al., 2011; **Table 1**).

The regenerative effects of MSCs largely depend on their capacity to regulate inflammation and tissue homeostasis via the secretion of an array of immunosuppressive factors, cytokines and growth and differentiation factors that may inhibit inflammatory responses and facilitate the proliferation and differentiation of progenitor cells in tissues *in situ*. P1 receptors are also involved in this aspect of MSC physiology following a pathological insult, being implicated in tissue repair and wound healing by stimulating local repair mechanisms and enhancing the accumulation of endothelial progenitor cells (Katebi et al., 2009). Released adenosine usually displays direct anti-inflammatory effects (Hasko and Pacher, 2008) blocking the proliferation of T-lymphocytes mainly through the A2A subtype, and the addition of A2A antagonists or CD39 inhibitors significantly counteracts this effect (Saldanha-Araujo et al., 2011; Sattler et al., 2011; Lee et al., 2014).

P2 Receptors have Pleiotropic Effects in MSCs

Human MSCs have been reported spontaneously to release ATP (Coppi et al., 2007) which, in a paracrine way, initiates and propagates intracellular Ca^{2+} waves, promoting the activation of transcription factors that are involved in cell differentiation (Kawano et al., 2006). ATP inhibits the proliferation of bone marrow (BM)-MSCs (Coppi et al., 2007) and stimulates their migration (Ferrari et al., 2011) and PPARγ levels through the activation of different P2X and P2Y receptor subunits (Omatsu-Kanbe et al., 2006; Zippel et al., 2012; Ciciarello et al., 2013; **Table 1**; **Figure 1B**). Together with this adipogenic role for extracellular nucleotides, it was recently demonstrated that P2 receptors are also involved in osteogenesis (**Table 1**) and up- or down-regulation of different P2 subtypes was observed in adipogenic and osteogenic differentiation of MSCs derived from adipose tissue and dental follicles (Zippel et al., 2012). In particular, P2Y13-deficient mice exhibit a decreased bone turnover associated with a reduction in the number of both osteoblasts and osteoclasts (Wang et al., 2014) and MSCs derived from these mice undergo a preferential adipogenic differentiation, showing that the P2Y13 receptor physiologically stimulates the differentiation of osteoblasts (**Figure 1B**) and inhibits that of adipocytes (Biver et al., 2013; **Table 1**). P2X7 receptor activation in BM-MSCs from post-menopausal women and following shockwave treatment also promotes osteogenic differentiation and mineralization (Sun et al., 2013; Noronha-Matos et al., 2014; **Table 1**; **Figure 1B**). Finally, it has been demonstrated that activation of P2Y11 receptor by NAD^+ released from connexin hemichannels increases proliferation, migration, and cytokine release in BM-MSCs, sparing in this case osteogenic and adipogenic differentiation markers (Fruscione et al., 2011; **Table 1**; **Figure 1B**).

Purinergic Ligands may be Involved in the Crosstalk between NSCs and MSCs

In this review we have described how purinergic signaling is involved in the physiology of NSCs and MSCs, as both cell types produce and respond to nucleotides and nucleosides. Although purinergic receptors can mediate different effects in the two cell niches (**Figure 1**), in both cases purinergic signaling converges

in the modulation of the immune response that is at the basis of stem cell recruitment, in particular after a stressful insult. The activation of P1 receptors is mainly immunosuppressive and trophic for stem cells, while the stimulation of P2 receptors is often proinflammatory and can enhance cell death pathways. Purinergic ligands produced and released by transplanted stem cells can behave as ideal candidates in promoting *in situ* cell growth and decreased apoptosis and in regulating inflammation. For example, although at present there is little evidence of transdifferentiation of MSCs into neurons, it is believed that the secretome of transplanted MSCs can empower surrounding cells to facilitate tissue repair also in CNS pathologies such as stroke, Parkinson's disease, traumatic brain injury, and epilepsy (Kim et al., 2009; Joyce et al., 2010). With regard to epilepsy, a large body of literature demonstrates the supporting role of adenosine as an endogenous anticonvulsant agent involved in anti-epileptic and anti-apoptotic functions, also by promoting neurogenesis (Glaser et al., 2012; Boison, 2013). Although numerous adenosine agonists have been shown to be potent anticonvulsants in a wide array of animal models of epilepsy, they often produce serious systemic adverse events. An alternative strategy under investigation is to transplant MSCs engineered to release high amounts of adenosine in several models of epilepsy, in order to enhance the natural adenosinergic mechanism triggered by seizures. This approach is very attractive as it provides large amounts of adenosine *in loco*, limiting its action to the foci of seizure and it has indeed proved successful, as engineered MSCs produce a local boost of adenosine and trigger anti-epileptic and anti-apoptotic effects (Boison, 2009; Li et al., 2009; Huicong et al., 2013).

In an acute optic nerve injury model it was shown that MSCs exert neuroprotective and anti-inflammatory effects, also through the down-regulation of the P2X7 receptor in retinal ganglion cells (Chen et al., 2013). Conversely, it was recently shown that ATP released from light-depolarized astrocytes promotes the neuronal differentiation of MSCs through the activation of P2X receptors *in vitro* and *in vivo* (Tu et al., 2014). It is evident from these results that purinergic ligands activate shared pathways that can be involved in MSC and NSC crosstalk, thus allowing mesenchymal and neurogenic niches to become closer.

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frontiers in Cellular Neuroscience

REVIEW
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Cellular targets for neuropeptide Y-mediated control of adult neurogenesis

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Neuropeptides are emerging as key regulators of stem cell niche activities in health and disease, both inside and outside the central nervous system (CNS). Among them, neuropeptide Y (NPY), one of the most abundant neuropeptides both in the nervous system and in non-neural districts, has become the focus of much attention for its involvement in a wide range of physiological and pathological conditions, including the modulation of different stem cell activities. In particular, a pro-neurogenic role of NPY has been evidenced in the neurogenic niche, where a direct effect on neural progenitors has been demonstrated, while different cellular types, including astrocytes, microglia and endothelial cells, also appear to be responsive to the peptide. The marked modulation of the NPY system during several pathological conditions that affect neurogenesis, including stress, seizures and neurodegeneration, further highlights the relevance of this peptide in the regulation of adult neurogenesis. In view of the considerable interest in understanding the mechanisms controlling neural cell fate, this review aims to summarize and discuss current data on NPY signaling in the different cellular components of the neurogenic niche in order to elucidate the complexity of the mechanisms underlying the modulatory properties of this peptide.

Keywords: neuropeptide Y, neurogenesis, neurogenic niche, neural stem cells, microglia, astrocyte, endothelium

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Introduction

In adult tissues, stem cells reside in a permissive and specialized microenvironment, or niche, in which different molecular signals coming from the external environment, together with feedback signals from progeny to parent cells, tightly regulate self-renewal, multipotency and stem cell fate (for review see Hsu and Fuchs, 2012). In this regard, many findings underlie the key role played by neurotransmitters on stem cell biology in niches located both inside and outside the central nervous system (CNS; for review see Katayama et al., 2006; Riquelme et al., 2008). Cross-species comparative analysis points out that it could be included in a more general and evolutionary old function, going beyond their role in inter-neuronal communication (for review see Berg et al., 2013). Among them, neuropeptides, molecules released both by neurons, as co-transmitters, and by many additional release sites (for review see van den Pol, 2012), are emerging as important mediators for signaling in both neurogenic and non-neurogenic stem cell niches (for review see Oomen et al., 2000; Louridas et al., 2009; Zaben and Gray, 2013), thus representing possible shared signaling molecules in their biological dynamics.

One of the most abundant neuropeptides in the CNS is neuropeptide Y (NPY), a 36-amino-acid polypeptide that is highly conserved during phylogenesis (Larhammar et al., 1993). Through its ability to modify its levels and expression pattern following environmental changes in both physiological and pathological conditions (Scharfman and Gray, 2006; Zhang et al., 2014), it is involved in many different functions, both inside and outside the CNS. These functions are performed by binding to different G-coupled NPY receptors distributed in different organs (Pedrazzini et al., 2003).

In peripheral organs, NPY can be found in sympathetic nerves, where its release mediates vasoconstrictive effects, in adrenal medulla and in platelets (for review see Hirsch and Zukowska, 2012). NPY takes part in cardiovascular and metabolic response to stress (for review see Hirsch and Zukowska, 2012), in coronary heart disease and hypertension (Zukowska-Grojec et al., 1993). More recently, the NPY-induced modulation of different stem cell niches has been highlighted. A direct role in adipogenesis has been indicated (Kuo et al., 2007; Park et al., 2014; Zhang et al., 2014), as well as its angiogenic properties, which have been widely described in different tissues (Ekstrand et al., 2003; Zukowska et al., 2003). The NPY system is also crucially involved in the regulation of the osteogenic niche, where its presence is due to both local production and release from NPY-immunoreactive fibers, and it plays a pivotal function in the neuro-osteogenic network that regulates bone homeostasis (Franquinho et al., 2010; Lee et al., 2010, 2011).

Within the CNS, NPY is a major regulator of food consumption and energy homeostasis (for review see Lin et al., 2004), acts as one of the crucial players of the stress-related mechanisms (for review see Hirsch and Zukowska, 2012), and participates in anxiety, memory processing and cognition (for review see Decressac and Barker, 2012). It is also involved in the pathogenesis of several neurologic diseases, including neurodegenerative diseases, such as Alzheimer's disease, Huntington's disease (revised by Decressac and Barker, 2012) and temporal lobe epilepsy (Marksteiner et al., 1989, 1990; Vezzani and Sperk, 2004), in which anticonvulsant and neuroprotective effects have also been observed (for reviews see Vezzani et al., 1999; Vezzani and Sperk, 2004; Gray, 2008; Decressac and Barker, 2012; Malva et al., 2012). At the cellular level, it is either co-released locally by GABAergic interneurons (for review see Sperk et al., 2007; Karagiannis et al., 2009) or comes from the blood by diffusion across the blood-brain barrier (Kastin and Akerstrom, 1999). It modulates excitatory neurotransmission and regulates hyperexcitability, particularly in the hippocampus (Baraban et al., 1997). The Y1, Y2 and Y5 receptors (Y1R, Y2R, Y5R) exhibit specific distribution patterns within the CNS (Parker and Herzog, 1999; Xapelli et al., 2006) and mediate the wide range of NPY physiological functions (Pedrazzini et al., 2003).

Due to the involvement of the NPY system in many of the numerous physiological (e.g., physical activity and learning), and/or pathological stimuli (e.g., stress, seizures, neurodegenerative diseases) (Redrobe et al., 2004; Vezzani and Sperk, 2004; Decressac and Barker, 2012; Hirsch and Zukowska, 2012; Jiang et al., 2014) that strictly regulate the biological dynamics of the neurogenic niche (Kempermann et al., 2004; Zhao et al., 2008), its role in the modulation of adult neurogenesis appears particularly relevant (for review see Gray, 2008; Decressac and Barker, 2012; Malva et al., 2012; Zaben and Gray, 2013).

Interestingly, NPY-responsive cells in the CNS are known as not being confined to neurons, but they also include astrocytes (Hösli and Hösli, 1993; Barnea et al., 1998; Ramamoorthy and Whim, 2008; Santos-Carvalho et al., 2013), oligodendrocyte precursor cells (Howell et al., 2007), microglia (Ferreira et al., 2010, 2011) and endothelial cells (Zukowska-Grojec et al., 1998), which are key components of the specialized microenvironment where adult neurogenesis takes place.

In this context, a comprehensive analysis of relevant data on the NPY-mediated control of adult neurogenesis, focusing on its effects on the different cellular components of the neurogenic niche, could be particularly helpful to improve our understanding of the complex functions of this neuropeptide.

NPY and Neural Stem Cells (NSCs)

The direct effects of NPY on neural elements of the different neurogenic niches located outside (olfactory epithelium [OE] and retina) or inside the CNS (subventricular zone [SVZ], subcallosal zone [SCZ], subgranular zone [SGZ]) have been widely studied (**Figure 1**). The proximity to anatomical elements releasing NPY and the stem cell expression of Y1R, as also described in the adipogenic and osteogenic niches (Togari, 2002; Lundberg et al., 2007; Lee et al., 2010; Zhang et al., 2014), are common elements.

Effects of NPY on the OE Niche

The vulnerability of olfactory sensory neurons to different environmental factors and the crucial role of the sense of smell in mammalian daily life account for neurogenesis in the OE; as the OE is accessible in living adult humans, it also offers a source of cells useful for understanding the biology of adult neurogenesis in health and disease (Mackay-Sim, 2010).

Hansel et al. provided the first evidence of a proliferative role of NPY on NSCs (namely basal cells) of the OE (Hansel et al., 2001), where the peptide is locally produced by the ensheathing cells of olfactory axon bundles and by sustentacular non-neuronal cells (Ubink et al., 1994).

Experiments performed using transgenic animals and primary olfactory cultures have shown that this effect is mediated by the Y1R (Hansel et al., 2001; Doyle et al., 2008) and involves Protein Kinase C and ERK1/2 pathways, which are ultimately involved in regulating the expression of genes involved in controlling cell proliferation and differentiation (Hansel et al., 2001). NPY release is regulated by ATP, which is constitutively expressed by the OE and preferentially released on injury, and the consequent activation of P2 purinergic receptors (Kanekar et al., 2009;

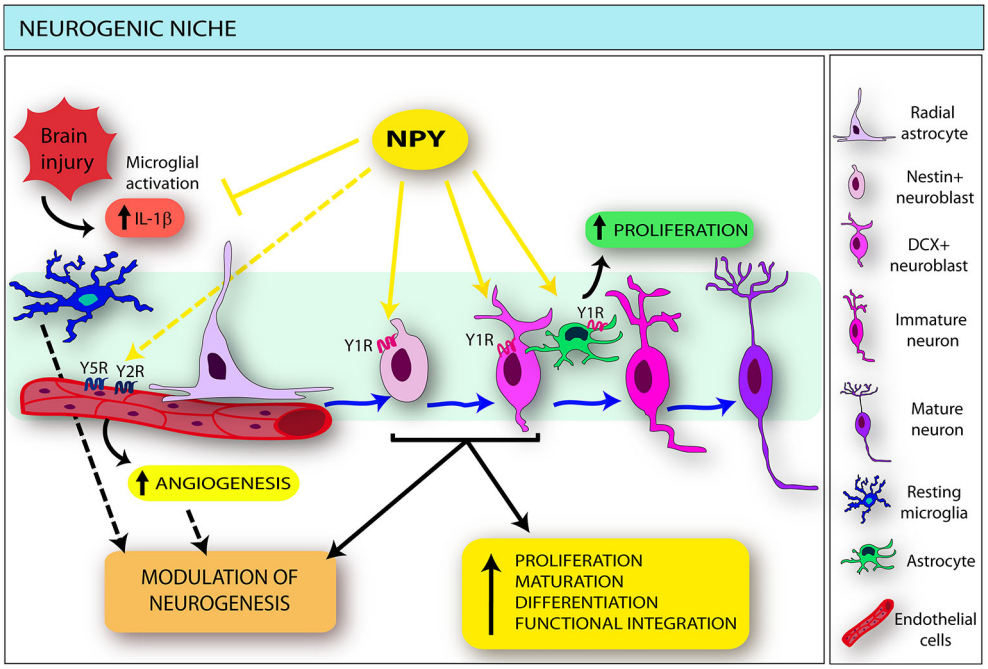

FIGURE 1 | Schematic drawing indicating the main effects exerted by neuropeptide Y (NPY) on the different components of the neurogenic niche. NPY, released by different sources in both physiological and pathological conditions, directly targets selected neural stem cell (NSC) subtypes (namely nestin- and doublecortin [DCX]-positive cells), inducing proliferation, differentiation, migration and functional integration of newly-born neurons. NPY also modulates microglia functions: through the interaction with the Y1R, it inhibits microglial activation and interleukin (IL)-1beta release. The influence of NPY-microglia interactions in the modulation of neurogenesis (dotted black arrow) may be hypothesized. In addition, NPY stimulates astrocyte proliferation mainly via the Y1 receptors (Y1R). NPY also acts on the endothelium through the Y2 receptors (Y2R), in cooperation with the Y5 receptors (Y5R): consequently a direct effect on the endothelial component of the neurogenic niche could be hypothesized (dotted yellow arrow), resulting in increased angiogenesis and possible modulation of endogenous neurogenesis (dotted black arrow).

Jia and Hegg, 2012). A role of NPY in the maturation and survival of olfactory receptor neurons has also been proposed (Doyle et al., 2012).

Effects of NPY on the Retinal Niche

Many findings suggest the presence of a regenerative potential within the mammalian retina, in which Muller astrocytes, that are responsible for the homeostatic and metabolic support of retinal neurons, appear capable of proliferating and giving rise to neuronal cells in response to retinal damage (for review see Lin et al., 2014). Both NPY and NPY receptors (Y1R, Y2R and Y5R) are expressed by the different retinal cellular subpopulations, namely neurons, astrocytes, microglia and endothelial cells (Alvaro et al., 2007; Santos-Carvalho et al., 2014). Interestingly, *in vitro* experiments in Muller cell primary cultures pointed out a modulatory role of NPY on cell proliferation: at low dose it negatively affects the proliferation rate of the cells, while at high doses it increases cell proliferation through the Y1R stimulation and consequent activation of the p44/p42 MAPKs, p38 MAPK and PI3K (Milenkovic et al., 2004). The NPY-mediated proliferative effect has been confirmed in experiments on retinal primary cultures, which revealed that NPY-treatment stimulates retinal neural cell proliferation, through nitric oxide (NO)-cyclic GMP and ERK 1/2 pathways via Y1R, Y2R and Y5R (Alvaro et al., 2008).

Effects of NPY on SGZ

Within the dentate gyrus (DG) NPY is selectively released by GABAergic interneurons located in the hilus, which innervate the granule cell layer in close proximity to the SGZ (for review see Sperk et al., 2007); a physiological role for NPY in the regulation of dentate neurogenesis can therefore be hypothesized. The pro-neurogenic role of NPY on hippocampal NSCs has been evidenced both *in vitro* (Howell et al., 2003, 2005, 2007) and *in vivo* (Decressac et al., 2011). *In vitro* evidence suggests a purely proliferative effect (Howell et al., 2007; Gray, 2008), specifically involving the Y1R, which is mediated by the intracellular NO pathway, through NO/cyclic guanosine monophosphate (cGMP)/cGMP-dependent protein kinase (Cheung et al., 2012), ultimately culminating in the activation of ERK1/2 signaling (Howell et al., 2003; Cheung et al., 2012). Interestingly, in line with the results obtained in the retinal niche (Alvaro et al., 2008), the role of NPY in the modulation of another signaling pathway driving a complex modulation of NSC activities emerges. It is well known, in fact, that NO exerts a dual influence on neurogenesis, depending on the source (for review see Carreira et al., 2012):

while intracellular NO is pro-neurogenic, the extracellular form exerts a negative effect (Luo et al., 2010). In this respect the Y1R has also been proposed as a key target in the selective promotion of NO-mediated enhancement of dentate neurogenesis (Cheung et al., 2012).

Decressac et al. confirmed, by *in vivo* administration of exogenous NPY in both wild type and Y1R knock out mice, that NPY-sensitive cells are the transit amplifying progenitors expressing nestin and doublecortin (DCX), which selectively express the Y1R (Decressac et al., 2011), as also evidenced *in vitro* (Howell et al., 2003; **Figure 1**). A preferential differentiation of newly generated cells towards a neuronal lineage has also been reported (Decressac et al., 2011). In this regard, it is worth emphasizing the role also played by NPY in seizure-induced dentate neurogenesis. Studies on NPY−/− mice show a significant reduction in bromodeoxyuridine incorporation in the DG after kainic acid administration (Howell et al., 2007). Interestingly, the DCX-positive cells, besides being selective targets of NPY, are one of the most important neuroblast subpopulations recruited in seizure-induced neurogenesis (Jessberger et al., 2005). These findings are in line with the notion that different neural progenitor subpopulations within the niche show different sensitivity to physiological and/or pathological stimuli (Kempermann et al., 2004; Fabel and Kempermann, 2008), thus representing selective targets for potential drugs aimed at modulating endogenous neurogenesis, of which NPY appears to be a possible candidate.

Exogenous NPY has been administered in the Trimethyltin (TMT)-induced model of hippocampal neurodegeneration and temporal lobe epilepsy, in which selective pyramidal cell loss in hippocampal CA1/CA3 subfields (Geloso et al., 1996, 1997), reactive astrogliosis and microglial activation (for review see Geloso et al., 2011; Corvino et al., 2013; Lattanzi et al., 2013) are associated with injury-induced neurogenesis (Corvino et al., 2005). NPY injection in TMT-treated rats results in long-term effects on the hippocampal neurogenic niche, culminating in the functional integration of newly generated neurons into the local circuit (Corvino et al., 2012, 2014). The early events following NPY administration are characterized by the up-regulation of genes involved in different aspects of NSC dynamics. In particular, *Noggin*, which participates in self-renewal processes (Bonaguidi et al., 2008), *Sox-2* and *Sonic hedgehog*, both involved in the establishment and maintenance of the hippocampal niche (Favaro et al., 2009), *NeuroD1*, which regulates differentiation and maturation processes (Roybon et al., 2009), *Doublecortin*, a driver of neuroblast migration (Nishimura et al., 2014) and *brain-derived neurotrophic factor* (BDNF), which is involved in different aspects of dentate neurogenesis (Noble et al., 2011), have all been reported to be significantly modulated within the first 24 h following treatment with NPY (Corvino et al., 2012, 2014). These findings suggest that *in vivo* NPY administration, in association with the peculiar changes in the microenvironment induced by the ongoing neurodegeneration, may trigger a complex mechanism that goes beyond a mere proliferative effect. It can be speculated that it occurs as the result of NPY's effect on both neural and non-neural elements of the niche and/or as a consequence of multiple cell-cell interactions (**Figure 2**).

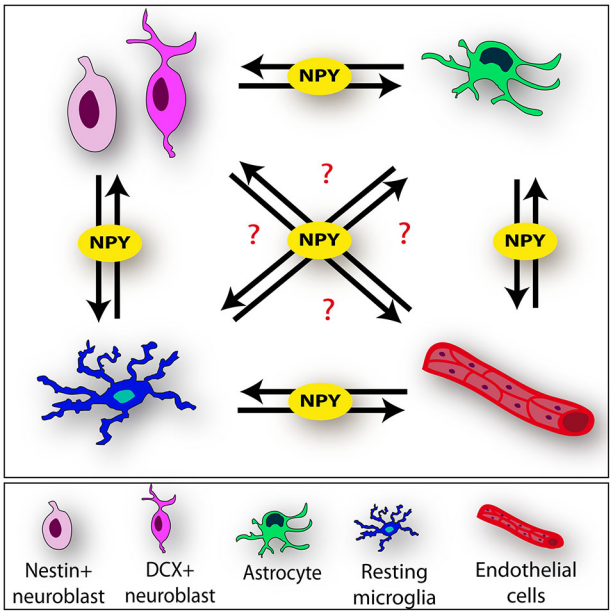

FIGURE 2 | Neuropeptide Y (NPY) mediates cell-cell interactions within the neurogenic niche. NPY may be involved as key player of the complex communication process among the different components of the niche (neural stem cell [NSCs], microglia, astrocytes and endothelium) (black arrows).

Effects of NPY on SVZ

In the SVZ, the most abundant reservoir of NSCs in the human brain (Doetsch, 2003b; Lim and Alvarez-Buylla, 2014), NPY comes from the cerebrospinal fluid, together with other nutrients and growth factors (Hou et al., 2006). Dense NPY-positive networks also surround this region (Stanic et al., 2008; Thiriet et al., 2011). NPY is also locally expressed by a subset of subependymal cells (Curtis et al., 2005) and by immature neural progenitors, thus suggesting a role as an autocrine/paracrine factor in the control of SVZ neurogenesis (Thiriet et al., 2011).

The effects of the peptide on the SVZ neurogenic niche have been assessed by both *in vitro* (Agasse et al., 2008; Thiriet et al., 2011) and *in vivo* studies (Stanic et al., 2008; Decressac et al., 2009). Also in this case the pro-neurogenic role of NPY is essentially played by the Y1R (Agasse et al., 2008; Stanic et al., 2008; Thiriet et al., 2011), which is mainly expressed by DCX-positive neuroblasts in adult mice (Stanic et al., 2008; **Figure 1**) and in Sox2 and nestin-positive cells in the developing rat (Thiriet et al., 2011). Consistently with the reported effects on dentate and olfactory NSCs, the Y1R mediates a proliferative effect, via phosphorylation of ERK MAP kinases p42 and p44 (Thiriet et al., 2011). The involvement of stress-activated protein kinase/JNK pathways, considered to play an important role in neural differentiation and maturation, has also been reported (Agasse et al., 2008).

It is well known that, while sharing common regulators, the different neurogenic niches may show some differences in specific aspects, including cellular organization, neuronal subtype differentiation and migration of NSCs (Ming and Song, 2011). In this regard, some discrepancies with the SGZ have

emerged: in the SVZ, in fact, NPY appears also to exert a direct role on cell migration (Decressac et al., 2009; Thiriet et al., 2011) and neuronal differentiation (Agasse et al., 2008; Decressac et al., 2009), while a mere proliferative role, without instructive signals to differentiation processes, emerged from *in vitro* studies on SGZ NSCs (Howell et al., 2007). In particular, *in vivo* administration of NPY in adult wild type mice showed that the newly generated neurons migrate not only to the olfactory bulb, but also towards the striatum, where they preferentially differentiate into GABAergic neurons (Decressac et al., 2009). Experiments performed on Y1R knock out mice indicated that they show a disrupted assembly of neuroblasts in the rostral migratory stream, compared with the chain-like organization present in wild type animals (Stanic et al., 2008), suggesting a role of this receptor also in cell migration. The direct demonstration of a chemokinetic effect of NPY through Y1R activation and MAPK ERK1/2 pathway recruitment in NSCs, was finally given by Thiriet et al. on rat SVZ neurospheres (Thiriet et al., 2011). The possible involvement of the Y2R has also been suggested, since Y2R null mice express a reduced number of migratory neuroblasts in both the SVZ and the rostral migratory stream, with a consequently reduced number of interneurons in the olfactory bulb (Stanic et al., 2008). It should be noted, however, that the Y2R protein was found only in close proximity to rostral migratory stream associated neuroblasts, without evidence of positivity in NSCs and/or astroglial cells (Stanic et al., 2008).

Many neurodegenerative diseases induce changes in SVZ neurogenesis (Curtis et al., 2007). Alzheimer's disease and Parkinson's disease, for instance, are accompanied by a reduction in NSC proliferation, while stroke and Huntington's disease cause an enhancement of SVZ neurogenesis, resulting in an increased number of new neurons, which also migrate into damaged areas (Curtis et al., 2007). Consequently, NPY administration may be of potential interest in cell replacement-based strategies for neurodegenerative diseases affecting SVZ neurogenesis. Decressac et al. demonstrated that NPY administration in the R6/2 model of Huntington disease is able to attenuate striatal atrophy and to induce a proliferative effect on SVZ NSCs (Decressac et al., 2010). However, it did not result in an increased number of newly generated neurons migrating within the striatum. NPY administration was also ineffective in modulating dentate neurogenesis in R6/2 mice. Interestingly, a reduced expression of NPY in the hilus of R6/2 mice was observed, accompanied by a reduction in the number of Y1R positive cells in the DG, thus suggesting that alterations in the NPY system might contribute to the impairment of neurogenesis in this model of Huntington disease (Decressac et al., 2010).

Effects of NPY on SCZ

NPY also exerts its proliferative role in the SCZ, a caudal extension of the SVZ lying between the hippocampus and the corpus callosum that, in basal conditions, essentially generates oligodendrocytes migrating into the corpus callosum (Seri et al., 2006). Acting through the Y1R on nestin-positive cells (Howell et al., 2007), NPY is involved in basal and seizure-induced SCZ progenitor cell proliferation (Howell et al., 2007; Laskowski et al., 2007). Interestingly, SCZ activity appears to be modulated by seizures, resulting in the production of glial progenitors that migrate to the injured hippocampus (Parent et al., 2006), thus raising the intriguing possibility that NPY modulates SCZ oligodendrogliogenesis as well as neurogenesis (Gray, 2008).

NPY and Microglia

Increasing evidence suggests that microglia play a relevant role in the neurogenic niche: unchallenged microglia contribute, through their phagocytic activity, to the maintenance of homeostasis of the neurogenic processes (Sierra et al., 2010), while the different functional phenotypic profiles that microglial cells undergo as a response to microenvironmental changes appear to have a dual role in neurogenesis (Carreira et al., 2012; Kettenmann et al., 2013; Su et al., 2014). Much evidence indicates how the pro-inflammatory cytokines released by activated microglia, such as interleukin (IL)-1beta, tumor necrosis factor (TNF)-alpha and IL-6, detrimentally affect neurogenesis (Ekdahl et al., 2003; Ekdahl, 2012; Su et al., 2014). On the other hand, in an enriched environment, activated microglia show proneurogenic properties via increased expression of insulin growth factor-1 (Ziv et al., 2006), while, in the presence of T-helper dependent cytokines, they reduce the production of TNF-alpha (Butovsky et al., 2006). In other words, the regulatory function of microglia in neurogenesis seems to be essentially dependent on differences in instructive signals coming from the microenvironment (Ekdahl et al., 2009).

Many studies support the modulatory role of NPY in the immune system, with effects ranging from the modulation of cell migration to macrophage and T helper cell differentiation, cytokine release, natural killer cell activity and phagocytosis, most likely through its Y1R (for review see Hirsch and Zukowska, 2012; Dimitrijević and Stanojević, 2013).

Recent findings also indicate direct interactions between NPY and microglia, the innate defensive system in the CNS (Kettenmann et al., 2013). Ferreira et al. observed that NPY, acting via the Y1R, inhibits lipopolysaccharide-induced microglial activation and reduces the associated release of IL-1beta (Ferreira et al., 2010). This effect is mediated by NPY-induced impairment of NO synthesis and reduced inducible form of nitric oxide synthase expression (Ferreira et al., 2010). In addition, NPY also induces impairment of the phagocytic properties of activated microglia (Ferreira et al., 2011) and IL-1beta-induced microglial motility (Ferreira et al., 2012). Taken together, these observations point to the key role played by the peptide in modulating the functional activities of microglia, and consequent release of mediators during inflammation (**Figure 1**).

Although most of these findings were obtained in *in vitro* systems, so that further research is needed in order to elucidate whether these interactions produce the same regulatory responses *in vivo*, a relevant influence of NPY-microglia interactions in the homeostasis of the neurogenic niche may be inferred. Because of the influence exerted by neuroinflammation on neurogenesis (Carreira et al., 2012),

NPY-microglia signaling could be particularly relevant in the modulation of injury-induced neurogenesis. Studies exploring the interaction between neuroinflammation and neurogenesis lead to the hypothesis that the early detrimental action of microglia after acute neuronal damage can, in some situations, be modified into a supportive state during the chronic phase (Ekdahl et al., 2009) and NPY could be involved in the modulation of these transient properties of activated microglia. Many findings emphasize the ability of NSCs to modulate their own environment through the release of signaling factors (Klassen et al., 2003; Butti et al., 2014) and mutual interaction between NSCs and microglia have been shown by recent research (Mosher et al., 2012). In this regard, we may speculate that NPY, released by NSCs or coming from the surrounding environment, could be critically involved in this process, acting as a paracrine/autocrine factor which modulates both the state of activation of microglial cells and their interactions with NSCs (**Figure 2**).

NPY and Astrocytes

Astrocytes are complex cells, whose supporting roles in the healthy CNS includes the regulation of blood flow, the modulation of synaptic function and plasticity and maintenance of the extracellular balance of ions and transmitters (Sofroniew, 2009). They also act as important regulators of the niche environment, through the secretion of diffusible factors (Lie et al., 2005; Barkho et al., 2006; Lu and Kipnis, 2010; Barkho and Zhao, 2011; Wilhelmsson et al., 2012) or through membrane-associated molecules (Barkho and Zhao, 2011). Thanks to their peculiar position between endothelial cells and neurons, astrocytes can mediate the exchange of molecules between vascular and neural compartments (Parpura et al., 2012). In addition, a specific subpopulation of astrocytes, the radial astrocytes, directly generates migrating neuroblasts, via rapidly dividing transit-amplifying cells (Seri et al., 2001; Doetsch, 2003a).

Several studies indicate that the expression of NPY and NPY receptors (namely Y1R) is also extended to some astrocyte subpopulations (Barnea et al., 1998, 2001; St-Pierre et al., 2000), including retinal astrocytes (Alvaro et al., 2007). It has been shown that astrocytes, like neurons, are able to synthesize NPY and show a regulated secretory pathway that is responsible for the release of multiple classes of transmitter molecules: in this regard, the activation of metabotropic glutamate receptors results in a calcium-dependent fusion of NPY-containing dense-core granules with the cell membrane and consequent peptide secretion (Ramamoorthy and Whim, 2008). It has been suggested that this process may be controlled by the RE-1–silencing transcription factor, the same factor that regulates neurosecretion in neurons (Prada et al., 2011). The expression of NPY in astrocytes is controlled by several factors: the post-natal down-regulation of glial peptide transcripts has been reported, as well as its up-regulation in adult astrocytes after brain injury (Ubink et al., 2003).

Interestingly, the *in vivo* intracerebroventricular administration of NPY significantly increases the proliferation not only of neuroblasts but also of astrocytes within the SVZ, mainly via the Y1R (Decressac et al., 2009; **Figure 1**). These findings delineate a complex scenario in which the peptide could exert its influence and, although direct evidence is still lacking, a role of NPY-gliotransmission in the modulation of critical steps of adult neurogenesis may be hypothesized, in both physiological and pathological conditions. In particular, it has been reported that the expression of astrocytic NPY also appears to be modulated in a cytokine-specific manner: in this regard, a relevant role of fibroblast growth factor (Barnea et al., 1998) and IL-beta (Barnea et al., 2001) in astrocytic NPY upregulation has emerged in *in vitro* studies. Both these factors can be released by astrocytes as well as by microglia: since, as previously reported, NPY inhibits microglial production of IL-1beta and IL-1beta-induced phagocytosis (Ferreira et al., 2011, 2012), a role of the peptide in astroglial/microglial interplay could be speculated. It is conceivable that it may be involved in the astrocytic regulation of microglial differentiation and activation, which, in turn, differently affect neurogenesis.

In addition, it has been reported that NPY increases the proliferative effect of the astrocyte-derived growth factor fibroblast growth factor-2 on NSCs, through the increased expression of fibroblast growth factor-receptor 1 on granule cell precursors (Rodrigo et al., 2010). This observation indicates the involvement of NPY also in the neuron-glial crosstalk and further reinforces the hypothesis that it could be one of the molecules significantly involved in the mutual interactions among the different components of the niche (**Figure 2**).

NPY and the Endothelium

The vasculature is a critical component of the neurogenic niche, and endothelial cells closely interact with NSCs to form "neurovascular niches", contributing to the regulation and maintenance of the niche (Palmer et al., 2000; Shen et al., 2004, 2008; Tavazoie et al., 2008; Goldberg and Hirschi, 2009; for review Goldman and Chen, 2011).

The molecular cross-talk between NSCs and endothelial cells is mediated by diffusible factors secreted by endothelial cells, such as BDNF and vascular endothelial growth factor (VEGF), as well as by cell-cell contact (Leventhal et al., 1999; Jin et al., 2002; Shen et al., 2004, 2008; Snapyan et al., 2009; Sun et al., 2010; for review Goldman and Chen, 2011; Vissapragada et al., 2014). Although the characterization of NPY receptors in the cerebral endothelium has not been fully clarified (Abounader et al., 1999; You et al., 2001), much evidence suggests that the endothelium could represent one of the sources, as well as one of the targets, of this peptide (Silva et al., 2005).

In this regard, different subtypes of human and rodent peripheral endothelial cells are now known to synthesize, store and constitutively express some elements of the NPY system, such as NPY itself, the Y1R and Y2R and the dipeptidyl peptidase IV, enzyme which converts NPY from the Y1R ligand to a selective agonist of Y2R (Loesch et al., 1992; Sanabria and Silva, 1994; Jackerott and Larsson, 1997; Zukowska-Grojec et al., 1998;

Ghersi et al., 2001; Lee et al., 2003a; Nan et al., 2004; Silva et al., 2005; Movafagh et al., 2006; Abdel-Samad et al., 2007). NPY also acts on the endothelium, promoting angiogenesis, mainly via the Y2R, in cooperation with the Y5R (Zukowska-Grojec et al., 1998; Zukowska et al., 2003; Ekstrand et al., 2003; Lee et al., 2003a; Pons et al., 2004; Movafagh et al., 2006). VEGF- and NO-dependent pathways are primarily involved (You et al., 2001; Chen et al., 2002; Lee et al., 2003b). The hypothesis that the endothelium may represent a non-neural store of NPY, where it acts in an autocrine and in a paracrine manner, has also been proposed (Silva et al., 2005).

The angiogenic action of NPY has been confirmed in several *in vitro* and *in vivo* models: using specific receptor antagonist or transgenic Y2R knockout mice, these studies reinforced the primary role of the Y2R in mediating NPY's angiogenic response (Zukowska-Grojec et al., 1998; Ghersi et al., 2001; Ekstrand et al., 2003; Lee et al., 2003a,b; Movafagh et al., 2006; **Figure 1**).

NPY also appears to exert a relevant role in the regulation and stimulation of angiogenesis in pathological processes and tissue repair, as evidenced in *in vivo* models of peripheral limb ischemia (Grant and Zukowska, 2000; Lee et al., 2003b; Tilan et al., 2013), skin wound repair (Ekstrand et al., 2003) and oxygen-induced retinopathy (Yoon et al., 2002), in which both exogenous and/or endogenous (released from neural and non-neural stores) NPY significantly contribute to tissue revascularization.

Angiogenesis and neurogenesis are related processes, as evidenced by data showing that cerebral endothelial cells activated by ischemia promote proliferation and differentiation of NSCs, while neural progenitor cells isolated from the ischemic SVZ promote angiogenesis (Teng et al., 2008). In this regard, it has also been shown that both angiogenesis and the expression of pro-angiogenic factors exert important functions in different stages of neurogenesis, such as proliferation, migration and survival (Jin et al., 2002; Louissaint et al., 2002). Interestingly, among these molecules, a relevant role is played by NO signaling, which regulates both angiogenesis and neurogenesis (Carreira et al., 2013), and whose activity is modulated by NPY not only in endothelial cells (You et al., 2001; Chen et al., 2002; Lee et al., 2003b), but also in NSCs (Cheung et al., 2012) and microglia (Ferreira et al., 2012).

It may be speculated that NPY, possibly released from the endothelium, acts as a diffusible factor that could influence and modulate elements of the neurovascular niche (**Figure 2**).

Concluding Remarks and Future Perspectives

In summary, existing data provide evidence that NPY modulates the neurogenic niche performing a pro-neurogenic role directly on the NSCs, while the possibility of a concomitant modulatory action on astrocytes, microglia and endothelium activities within the niche is also possible. The involvement of NPY as a key player in the complex process of communication among the different components of the niche may be speculated, and, in this regard, there is evident need for further research to definitely elucidate the mechanisms of NPY-modulated cell/cell interactions. This could yield a more heightened understanding of some critical steps of the complex mechanisms that regulate adult neurogenesis, thus possibly providing knowledge useful to identify selective targets for potential drugs aimed at modulating NSC fate. Moreover, due to the significant involvement of the NPY system also in non-neural stem cell niches, this information could contribute to clarify the systemic role of the peptide, which appears to be involved in a set of basic homeostatic body functions, ranging from food consumption and energy homeostasis to the regulation of stem cell biology in adult tissues.

Authors and Contributors

MCG: She gave substantial contributions to both the conception and design of the work; she contributed to the acquisition, analysis, and interpretation of data. She drafted the work and revised it critically. She gave the final approval of the version to be published. She agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

VC: She gave substantial contributions to the design of the work; she contributed to the acquisition, analysis, and interpretation of data for the work. She drafted the work and revised it critically. She gave the final approval of the version to be published. She agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

VDM: She contributed to the acquisition of data for the work. She drafted the work. She gave the final approval of the version to be published. She agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

EM: She contributed to the acquisition of data for the work. She drafted the work. She gave the final approval of the version to be published. She agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

FM: He provided substantial contributions to the design of the work; he contributed to the interpretation of data for the work. He revised critically the work. He gave the final approval of the version to be published. He agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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REVIEW
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Sympathoadrenergic modulation of hematopoiesis: a review of available evidence and of therapeutic perspectives

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Innervation of the bone marrow (BM) has been described more than one century ago, however the first *in vivo* evidence that sympathoadrenergic fibers have a role in hematopoiesis dates back to less than 25 years ago. Evidence has since increased showing that adrenergic nerves in the BM release noradrenaline and possibly also dopamine, which act on adrenoceptors and dopaminergic receptors (DR) expressed on hematopoietic cells and affect cell survival, proliferation, migration and engraftment ability. Remarkably, dysregulation of adrenergic fibers to the BM is associated with hematopoietic disturbances and myeloproliferative disease. Several adrenergic and dopaminergic agents are already in clinical use for non-hematological indications and with a usually favorable risk-benefit profile, and are therefore potential candidates for non-conventional modulation of hematopoiesis.

Keywords: dopamine, noradrenaline, adrenaline, adrenoceptors, dopaminergic receptors, hematopoiesis, neuroimmune phamacology, drug repurposing

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Introduction

The term "niche", derived from the Latin word "mytilus" (mussel), has eventually come to designate a shallow recess in a wall, as for a statue or other decorative object, in view of the similarity with the shape of a seashell, and broadly a place suitable or appropriate for a person or thing. In biology and medicine, the use of "niche" to designate the microenvironment where cells are found, and which may determine their fate, becomes increasingly popular in the early 90's of the last century, thereafter steadily rising, from 27 papers/year on average in the period 1991–2000 (including about 3, 4 dealing with stem cells) to more than 1000/year since 2011 (about two thirds of them dealing with stem cells; **Figure 1**). So far, niches for several types of stem cells have been identified and characterized, including neurogenic (Bjornsson et al., 2015), osteogenic (Bianco, 2011), epithelial (Secker and Daniels, 2009), hematopoietic (Mendelson and Frenette, 2014).

The hematopoietic stem cell (HSC) niche as an organized microenvironment that controls HSC homeostasis was first proposed in Schofield (1978) and thereafter much progress has been made in characterizing the different cell types that are essential in HSC maintenance and regeneration (Lymperi et al., 2010; Wang and Wagers, 2011; Mendelson and Frenette, 2014), including perivascular stromal cells, reticular cells, endothelial cells, macrophages as well as sympathoadrenergic nerve terminals.

Sympathetic fibers innervating the bone marrow (BM) were described at least 70 years ago (Kuntz and Richins, 1945), their stimulation resulting in the release of reticulocytes and neutrophils into systemic circulation (DePace and Webber, 1975), however for many years their

role was mainly related to the regulation of the permeability of the venous sinusoids and the mobility of BM cells, until the evidence was provided that chemical sympathectomy increases the number of peripheral blood leukocytes after syngeneic BM transplantation in mice, an effect which is mimicked by the α_1-adrenoceptor antagonist prazosin (Maestroni et al., 1992). Nowadays, sympathetic nerves are considered, together with the hypothalamus-pituitary-adrenal axis, the main communication pathway between the brain and the immune system (Elenkov et al., 2000; Marino and Cosentino, 2013) and about one hundred papers have been published dealing with adrenergic modulation of hematopoiesis (**Figure 1**). It appears therefore that, despite sympathetic innervation of the BM has been known for decades, sympathoadrenergic modulation of hematopoiesis involves so far relatively few scientists around the world, a somewhat paradoxical observation in view of the many significant therapeutic opportunities which could arise from this field of research.

We will hereafter review current knowledge on innervation of the BM and on sympathoadrenergic modulation of hematopoiesis, discussing available evidence in light of the opportunity to repurpose adrenergic (and possibly also dopaminergic) agents as modulators of hematopoiesis. Indeed, any dirrectly and indirectly acting adrenergic and dopaminergic therapeutics are currently used for non-hematological indications, and could thus represent an attractive source of non-conventional agents for the modulation of the hematopoietic process. To this end, a brief general introduction to the neuroimmune pharmacology of catecholamine neurotransmitters will be first provided.

Neuroimmune Pharmacology of Catecholamine Neurotransmitters

Noradrenaline is a neurotransmitters in the central and peripheral nervous systems, and to a lesser extent a neurohormone in chromaffin cells in medulla of adrenal glands. From the *locus coeruleus* (LC), axons project rostrally, dorsally, and caudally to spinal cord, affecting attention, arousal and vigilance, and regulating hunger and feeding behavior. Adrenaline is a minor neurotransmitter in the central nervous system (CNS), however it is the main neurohormone secreted by the adrenal medulla. In periphery, noradrenaline is the main transmitter of sympathetic postganglionic fibers. Peripheral adrenergic actions include: smooth muscles contraction (skin, kidney, and mucous membranes blood vessels), stimulation of sweat glands, relaxation gut wall, bronchi, skeletal muscle blood vessels, increases of heart rate and contraction force. In addition, they have prominent metabolic (increased liver and muscle glycogenolysis, increased lipolysis) and endocrine actions (e.g., modulation of insulin and renin secretion). Dopamine is a key neurotransmitter in the brain, where it is involved in a wide variety of CNS functions including motivation, cognition, movement and reward. Besides being biochemically and metabolically related (since are all produced from the non-essential amino acid tyrosine; **Figure 2**), several lines of evidence suggest that dopamine may be stored in and released from sympathetic nerve terminals, thus acting as a transmitter even at this level (Bell, 1988; Bencsics et al., 1997). Detailed discussion of dopamine, noradrenaline and adrenaline neurochemistry, anatomy and physiology can be found in Feldman et al. (1997).

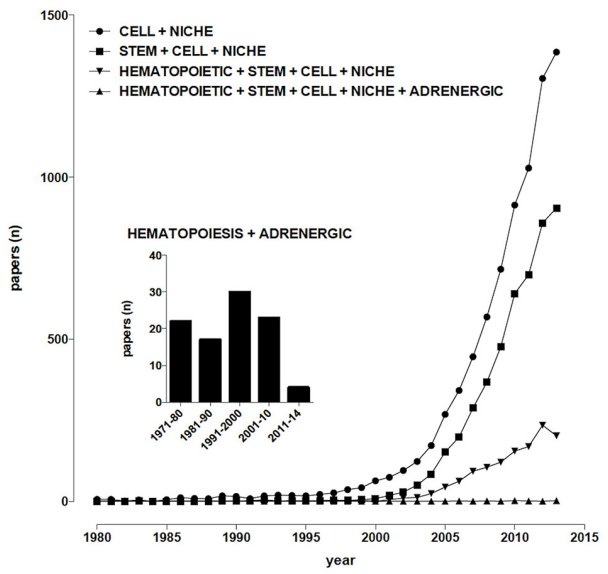

FIGURE 1 | Temporal trends of papers indexed in PubMed. (Alexandru Dan Corlan. Medline trend: automated yearly statistics of PubMed results for any query, 2004. Web resource at URL: http://dan.corlan.net/medline-trend.html. Accessed: 2015-03-24. Archived by WebCite at http://www.webcitation.org/65RkD48SV).

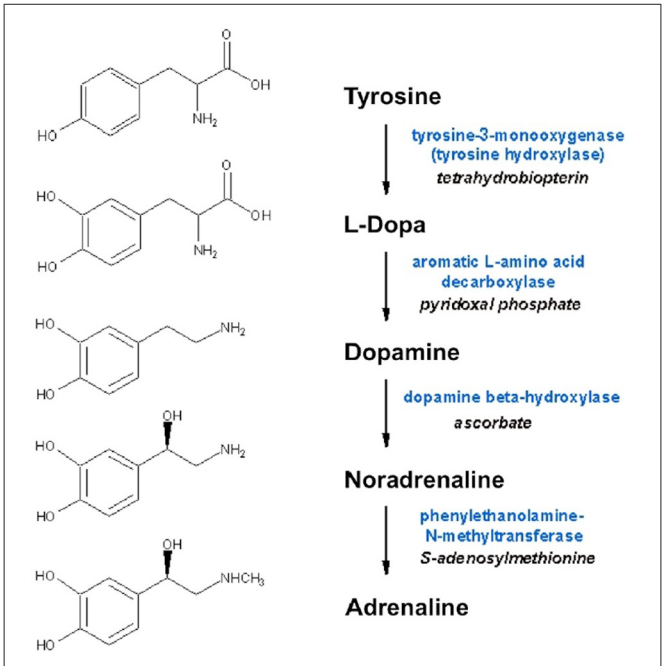

FIGURE 2 | Biosynthesis of dopamine, noradrenaline and adrenaline. Synthesizing enzymes and enzyme cofactors are shown close to each arrow.

Pharmacology of Dopamine, Noradrenaline and Adrenaline

Dopamine, noradrenaline and adrenaline act on 7-transmembrane, G-protein coupled receptors. Dopaminergic receptors (DR) exist in five different molecular subtypes, grouped into two families according to their pharmacology and second messenger coupling: the D_1-like (D_1 and D_5) activating adenylate cyclase and the D_2-like (D_2, D_3 and D_4) inhibiting adenylate cyclase (Beaulieu and Gainetdinov, 2011; Alexander et al., 2013; Cosentino et al., 2013). Adrenoceptors (ARs) are nine different receptors, including three major types—α_1, α_2 and β—each further divided into three subtypes (Alexander et al., 2013). DR agonists are used to treat Parkinson's disease (PD), restless leg syndrome, and hyperprolactinemia, while antagonists are used as antipsychotics and antiemetics (**Table 1**). AR agonists and antagonists are used to treat hypertension, angina pectoris, congestive heart failure, asthma, depression, benign prostatic hypertrophy, and glaucoma, as well as other conditions such as shock, premature labor and opioid withdrawal, and as adjunct medications in general anaesthesia (**Table 2**). Pharmacological modulation of adrenergic and dopaminergic pathways can be obtained also by use of indirectly acting agents. All the steps involved in dopamine, noradrenaline and adrenaline synthesis, storage and release, uptake and metabolism represent the target of several drugs already in use for non-immune indications (e.g., cardiovascular, neurologic, neuropsychiatric). Pharmacological targets and examples of therapeutic drugs are listed in **Tables 3** and **4** (Cosentino et al., 2013).

Adrenergic Pathways in the Modulation of the Immune Response

The two major pathway are involved in the brainimmune cross-talk are the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system. The role of the sympathetic nervous system in the neuroimmune crosstalk has been the subject of several reviews (Elenkov et al., 2000; Nance and Sanders, 2007; Flierl et al., 2008; Cosentino and Marino, 2013; Marino and Cosentino, 2013). The predominant view includes the release of noradrenaline by sympathoadrenergic terminals, followed by activation of β_2-ARs finally resulting into antiinflammatory effects (including to a variable extent the inhibition of T helper (Th) 1 proinflammatory cytokines such as IFN-γ, IL-12, TNF-α, and the enhancement of Th2 cytokines such as IL-10 and and transforming growth factor, TGF-β). Notably however noradrenaline may also promote IL-12-mediated differentiation of naive CD4+ T cells into Th1 effector cells which eventually produce IFN-γ (Swanson et al., 2001; Cosentino et al., 2013). Although β-ARs are considered the main interface between sympathoadrenergic terminals and immune cells, α-ARs may also occur in immune cells where they elicit proinflammatory responses, as in the case of α_1-ARs on human macrophages (Grisanti et al., 2011) and of α_2-ARs on rodent phagocytes (Flierl et al., 2007).

Dopaminergic Pathways in the Modulation of the Immune Response

In comparison to noradrenaline and adrenaline, the immune effects of dopamine emerged only recently but very quickly attracted increasing attention (reviewed in Basu and Dasgupta, 2000; Sarkar et al., 2010; Levite, 2012). DR are expressed in most if not all human immune cells, including T and B cells, dendritic cells, macrophages, microglia, neutrophils and NK cells, and immune cells can "meet" dopamine not only in brain but also in blood, lymphoid organs and in several other peripheral tissues, such as the kidney and the hepatic vasculature (reviewed by Levite, 2012; Cosentino et al., 2013). Among human immune cells, $CD4+CD25^{high}$ T lymphocytes are specifically sensitive to the activation of D_1-like receptors expressed on their membrane, resulting in inhibition of the regulatory functions of this specialized cell subset, which usually suppresses the activity of effector T cells (Cosentino et al., 2007). Dopamine is also an emerging regulator of dendritic cell and T cell physiology, with critical implications for onset of immune-related disorders (Pacheco et al., 2009).

Immune Cells as a Source of Dopamine, Noradrenaline and Adrenaline

Several types of immune cells may produce store and utilize catecholamines as autocrine/paracrine transmitters. The synthesis of dopamine, noradrenaline and adrenaline in immune cells likely occurs by means of a classical pathway, as suggested

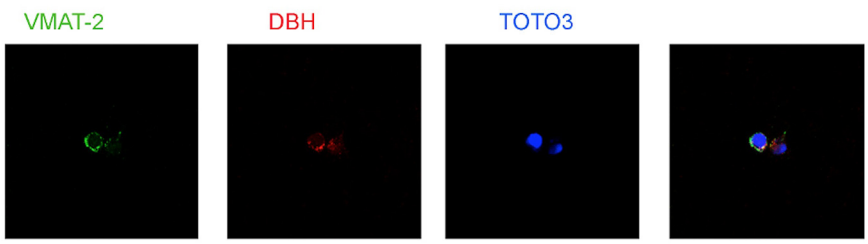

FIGURE 3 | Expression of vesicular monoamine transporters (VMAT2) and Dopamine β-hydroxylase (DBH) in human peripheral blood mononuclear cells (PBMCs). TOTO-3 iodide (642/660) was used for staining of nuclei. Cells were prepared at the Center for Research in Medical Pharmacology, University of Insubria, Varese (I) and analysis was performed at the Consorzio MIA—Microscopy and Image Analysis, Faculty of Medicine, University of Milan Bicocca, Milan (I).

TABLE 1 | Examples of dopaminergic agonists and antagonists currently used as therapeutic drugs (brand names in parentheses).

Agonists	
D_1-like	Fenoldopam mesylate (Corlopam)
D_1-like/D_2-like	Ergot Alkaloids: bromocriptine (Parlodel); pergolide (Permax); cabergoline (Dostinex) Non-Ergot Alkaloids: apomorphine (Apokyn); rotigotine (Neupro)
D_2-like	Non-Ergot Alkaloids: pramipexole (Mirapex); ropinirole (Requip)
Antagonists	
	Typical antipsychotics chlorpromazine (Thorazine), fluphenazine (Prolixin), haloperidol (Haldol), loxapine (Loxitane), molindone (Moban), perphenazine (Trilafon), pimozide (Orap), thioridazine (Mellaril), thiothixene (Navane), trifluoperazine (Stelazine)
	A typical antipsychotics amisulpride (Solian), clozapine (Clozaril), olanzapine (Zyprexa), quetiapine (Seroquel), risperidone (Risperdal), sulpiride (Dogmatil), ziprasidone (Geodon)
	Antiemetics domperidone, metoclopramide (Reglan), prochlorperazine (Compazine)

by the presence of the enzyme tyrosine hydroxylase (TH, EC 1.14.16.2), the first and rate-limiting enzyme in the synthesis of catecholamines, which undergoes upregulation following cell stimulation. TH inhibition, e.g., by α-methyl-*p*-tyrosine, prevents intracellular enhancement of catecholamines (Musso et al., 1996; Bergquist and Silberring, 1998; Cosentino et al., 1999, 2002a,b; Marino et al., 1999; Reguzzoni et al., 2002). In human peripheral blood mononuclear cells (PBMCs) stimulated *in vitro* with with phytohemagglutinin (PHA), TH mRNA expression and catecholamine production occur only in T and B lymphocytes (but not in monocytes) and are reduced by dopaminergic D_1-like receptor activation (Ferrari et al., 2004), as well as by the proinflammatory cytokine IFN-γ, which in turn is counteracted by IFN-β (Cosentino et al., 2005). Human lymphocytes possess reserpine-sensitive compartments and vesicular monoamine transporters (VMAT) which are involved in intracellular storage of catecholamines (Marino et al., 1999; Cosentino et al., 2000, 2007; **Figure 3**). Catecholamine release can be induced by biological agents such as IFN-β (Cosentino et al., 2005) or by elevation of extracellular K^+ ($[K^+]_e$; Cosentino et al., 2003). Human lymphocytes also express membrane transporter for dopamine (DAT; Marino et al., 1999; Marazziti et al., 2010) and for noradrenaline (NET; Audus and Gordon, 1982).

Innervation of the BM and of other Hematopoietic Organs and Tissues

Primary lymphoid organs, such as BM and thymus, as well as secondary lymphoid organs, such as spleen and lymph nodes, are innervated by autonomic sympathoadrenergic efferent nerve fibers. The sympathetic nervous system and the hypothalamic-pituitary-adrenal axis are the major pathway connecting the CNS and the immune system (reviewed in Elenkov et al., 2000). Several excellent reviews discuss in detail the origin, distribution, signaling and targets of sympathetic nerves in lymphoid organs (Felten et al., 1985; Felten and Felten, 1988; Felten, 1991; Straub, 2004), the effect of age (Bellinger et al., 1992; Madden et al., 1995, 1997, 1998; Friedman and Irwin, 1997) and stress (Irwin, 1994; Marshall and Agarwal, 2000; Nagatomi et al., 2000; Sloan et al., 2008) as well as the relevance of dysregulated sympathetic nerovus system in immune-mediated disease (Bellinger et al., 1992, 2008; Madden et al., 1995; Friedman and Irwin, 1997; Marshall and Agarwal, 2000; Frohman et al., 2001; Straub et al., 2006; Wrona, 2006; del Rey and Besedovsky, 2008; Benarroch, 2009).

Sympathoadrenergic Modulation of Hematopoiesis

Until the early 80 s, interest on adrenergic regulation of BM function was essentially concentrated on erythropoiesis (see e.g., Beckman et al., 1980; Lipski, 1980; Mladenovic and Adamson, 1984), with a few work dedicated to thrombocytopoiesis (Ganchev and Negrev, 1989).

Maestroni et al. (1992) were the first describing adrenergic modulation of hematopoiesis in an *in vivo* model, showing that chemical sympathectomy by 6-hydroxydopamine (6-OHDA) significantly increased the number of peripheral blood leukocytes after syngeneic BM transplantation in mice, an effect which was mimicked by the α_1-AR antagonist prazosin. Results were reproduced in normal mice (Maestroni and Conti, 1994), by showing that prazosin can also enhance myelopoiesis and platelet formation, while noradrenaline and the α_1-adrenergic agonist methoxamine could directly inhibit the *in vitro* growth of granulocyte/macrophage-colony-forming unit (GM-CFU). The order of potency of α-adrenergic antagonists on the effect of noradrenaline was prazosin>phentolamine>yohimbine. On these basis, the authors suggested that prazosin binds specifically to both BM cell membranes and intact BM cells, on

TABLE 2 | Examples of dopaminergic agonists and antagonists currently used as therapeutic drugs.

α_1AR	
Agonists	Methoxamine, methylnorepinephrine, midodrine, oxymetazoline, metaraminol, phenylephrine
Indications	*Vasoconstriction and mydriasis, used as vasopressors, nasal decongestants and eye exams*
Antagonists	Alfuzosin, doxazosin, phenoxybenzamine, phentolamine, prazosin, tamsulosin, terazosin, trazodone
Indications	*Hypertension, benign prostatic hyperplasia*
α_2-AR	
Agonists	Dexmedetomidine, medetomidine, romifidine, clonidine, brimonidine, detomidine, lofexidine, xylazine, tizanidine, guanfacine, amitraz
Indications	*Antihypertensives, sedatives and treatment of opiate dependence and alcohol withdrawal symptoms*
Antagonists	Phentolamine, yohimbine, idazoxan, atipamezole, trazodone, mianserin, mirtazapine
Indications	*Aphrodisiac, antidepressants, reversal of α_2-AR agonist-induced sedation*
β-AR **β_1-AR**	
Agonists	Dobutamine, isoprenaline, noradrenaline
Indications	*Bradycardia, heart failure, cardiogenic shock*
Antagonists	Metoprolol, atenolol, bisoprolol, propranolol, timolol, nebivolol
Indications	*Cardiac arrhythmia, congestive heart failure, glaucoma, myocardial infarction, migraine prophylaxis*
β_2-AR	
Agonists	Short-acting: salbutamol, levosalbutamol, terbutaline, pirbuterol, procaterol, clenbuterol, metaproterenol, fenoterol, bitolterol mesylate, ritodrine, isoprenaline. Long-acting: salmeterol, formoterol, bambuterol, clenbuterolUltra-long-acting: indacaterol
Indications	*Asthma (effects: dilation of bronchial passages, vasodilation in muscle and liver, relaxation of uterine muscle, and release of insulin)*
Antagonists	Butoxamine, timolol, propranolol
Indications	*Glaucoma, heart attacks, hypertension, migraine headache; investigational: stage fright, PTSD*
β_3-AR	
Agonists	Amibegron (*investigational*: *antidepressant, anxiolytic*), solabegron (*overactive bladder, irritable bowel syndrome*)
Antagonists	SR 59230A

two distinct binding sites, one with a K_d of 0.98 ± 0.32 nM and a B_{max} of 5 ± 2.9 fM/2 × 10^6 cells (higher affinity site), and another with a K_d of 55.9 ± 8.2 nM and a B_{max} of 44 ± 7.7 fM/mg protein. Several lines of evidence suggest that the higher affinity site is actually an α_1-AR, while the low affinity binding site remains to be characterized. The high-affinity binding is due to a lymphoid/stem cell fraction with no blasts and no GM-CFU progenitors, while the low-affinity site was apparent on a fraction enriched with GM-CFU progenitor cells (Maestroni and Conti, 1994). An initial summary of the significant evidence so far provided was published in Maestroni (1995), emphasizing the ability of α-AR antagonists to enhance myelopoiesis and platelets production while decreasing lymphopoiesis, in both normal mice as well as after BM transplantation. AR agonists, like the sympathetic neurotransmitter noradrenaline, seem to inhibit myelopoiesis, and effect which might be of clinical relevance, since it rescues the blood forming system and improves the survival of mice injected with a lethal dose of carboplatin or exposed to X-ray irradiation. This effect is apparently mediated by activation of α_1-ARs expressed in pre-B cells, in turn inducing the production of TGF-β, which is finally responsible for the haematopoietic effects (Maestroni, 1995). Remarkably, it has been recently shown that nonmyelinating Schwann cells, which ensheath autonomic nerves in the BM, maintain HSC dormancy by activating latent TGF-β and that glial cell death and loss of HSC result from autonomic denervation of BM (Yamazaki et al., 2011). Noradrenaline was most effective at 3 mg/kg, s.c., and protected 77% of the mice injected i.v. with 200 mg/kg of carboplatin, which has a LD_{100} of 170 mg/kg. The effects was profoundly antagonized by the α_1-AR antagonist prazosin. *In vitro*, 1 μM noradrenaline rescued GM-CFU in unseparated BM cells containing the adherent population expressing the high affinity α_1-AR, another effect which was consistently counteracted by low concentrations of the α_1-AR antagonist prazosin (0.1 nM-10 nM; Togni and Maestroni, 1996). Such results apparently challenge early reports suggesting that *in vitro* the β-AR agonist isoproterenol might result in increased proliferation and sensitivity of HSC to cytotoxic agents, an effect which was inhibited by the β-AR antagonist propranolol (Byron, 1972), however the studies cannot be directly compared due to fundamental differences in the experimental models and in the pharmacological agents employed. Interestingly, it was

TABLE 3 | Pharmacological targets for the modulation of dopaminergic and adrenergic pathways by agents targeting storage and release (brand/street names in parentheses).

Reuptake inhibitors/transporter blockers	
DAT inhibitors	Methylphenidate (Ritalin, Focalin, Concerta), bupropion (Wellbutrin, Zyban), amineptine (Survector, Maneon, Directin), nomifensine (Merital, Alival), cocaine, methylenedioxypyrovalerone (MDPV; "Sonic"), ketamine (K; Ketalar, Ketanest, Ketaset; "Special-K", "Kit Kat", etc.), phencyclidine (PCP; Sernyl; "Angel Dust", "Rocket Fuel", etc.)
Indications	*Attention-deficit hyperactivity disorder (ADHD), narcolepsy, obesity as anorectics, depression and anxiety, drug addiction, sexual dysfunction, illicit street drugs*
VMAT2 inhibitors	Reserpine (Serpasil), tetrabenazine (Nitoman, Xenazine), deserpidine (Harmonyl)
Indications	*Sympatholytics or antihypertensives, antipsychotics*
Releasing agents	Amphetamine (Adderall, Dexedrine; "Speed"), lisdexamfetamine (Vyvanse), methamphetamine (Desoxyn; "Meth", "Crank", "Crystal", etc.), methylenedioxymethamphetamine (MDMA; "Ecstasy", "E", "X", "XTC", etc.), phenmetrazine (Preludin; "Prellies"), pemoline (Cylert), 4-methylaminorex (4-MAR; "Ice", "Euphoria", etc.), benzylpiperazine (BZP; "Bennies", "A2", "Sunrise", "Frenzy", etc.)
Indications	*Attention-deficit hyperactivity disorder (ADHD), narcolepsy, obesity, depression and anxiety, drug addiction, sexual dysfunction, illicit street drugs*
"Activity enhancers"	Benzofuranylpropylaminopentane (BPAP), phenylpropylaminopentane (PPAP)
Indications	*Investigational*: *Alzheimer's disease, Parkinson's disease and clinical depression*

DAT, dopamine transporter; VMAT2, vesicular monoamine transporter type 2.

recently shown that also dopamine (50 mg/kg/days × 7 days i.p.), besides inhibiting tumor angiogenesis and growth of HT29 human colon cancer and Lewis lung carcinoma (LLC) in mice, also did not cause hypertension, hematological, renal and hepatic toxicities in normal, HT29 and LLC tumor bearing animals, and also prevented 5-fluorouracil (5FU) induced neutropenia in HT29 colon cancer bearing mice, an action apparently mediated through inhibition of 5FU mediated suppression of GM-CFU in the BM (Sarkar et al., 2014). In subsequent studies (Maestroni et al., 1997), it was further confirmed that noradrenaline administration in mice rescued hematopoiesis from the toxic effect of the chemotherapeutic agent carboplatin administered at supralethal doses (200 mg/kg), possibly by protecting GM-CFU. Meanwhile, Afan et al. (1997) reported that denervation decreases femoral cellularity as well as progenitor cells while mobilizing these cells in the peripheral blood of splenectomized mice. In non splenectomized animals, these changes were quickly cleared (Afan et al., 1997).

The consistent effects of noradrenaline and dopamine in the BM raised immediately the question regarding their physiological relevance and in particular the origin of catecholamines at this level. By use of a high performance liquid chromatographic method, we therefore measured endogenous catecholamines in BM from normal, 6-OHDA-treated and pargyline-treated mice. Noradrenaline levels were lower after 6-OHDA and higher after pargyline, while adrenaline and dopamine were not affected in either conditions (Marino et al., 1997). In the BM however noradrenaline, as well as the other catecholamines dopamine and adrenaline, may originate not only from nerve fibers but also from hematopoietic and immune cells themselves (Maestroni et al., 1998). In particular, in murine BM we described a daily rythmicity for noradrenaline and dopamine, with peak values occurring at night. Chemical sympathectomy disrupted the rhythm, whereas adrenaline showed no rhythmicity or 6-OHDA sensitivity. Noradrenaline was also positively associated with the proportion of cells in the G2/M and S phases of the cell cycle. Remarkably, in Méndez-Ferrer et al. (2008) published an elegant article suggesting just the opposite, i.e., that noradrenaline release in mouse BM is higher during the day/light hours. However, the findings of Maestroni et al. (1998) cannot be compared directly with those of Méndez-Ferrer et al. (2008) because the latter did not measure catecholamine concentration in the BM as Maestroni et al. did. The circadian release of noradrenaline was inferred by indirect experiments such as denervation, use of gene knock-out mice, and the catecholamine function was mimicked by injection of adrenergic agonists and/or antagonists. In addition, Maestroni et al. (1998) showed that BM cells themselves do contain catecholamines, therefore catecholamines in the BM resulted from both neural and hematopoietic cell contribution. Hence, Méndez-Ferrer et al. (2008) detected only one component of the system that was related to HSC trafficking while Maestroni et al. (1998) found a correlation between noradrenaline and BM cell proliferation. However, both groups found that chemical sympathectomy by 6-OHDA abolished the rhythm. Thus, a possible hypothetical interpretation that might reconcile these divergent findings is that the light/dark rhythm synchronizes the suprachiasmatic nucleus in the CNS which, in turn, entrains the sympathoadrenergic rhythm in the BM regulating the HSC traffic. In addition, the very same sympathetic nervous system or other circadian signals might affect clock genes in hematopoietic cell progenitors, influencing their noradrenaline content and their proliferation. Consistently, it has been reported that noradrenaline may affect clock genes expression (Morioka et al., 2010). Another circadian signal that ensues at the beginning of the activity

TABLE 4 | Pharmacological targets for the modulation of dopaminergic and adrenergic pathways by agents targeting metabolism (brand/street names in parentheses).

Reuptake inhibitors/transporter blockers	
Monoamine oxidase inhibitors	Nonselective agents: phenelzine (Nardil), tranylcypromine (Parnate), isocarboxazid (Marplan)MAOA selective agents: moclobemide (Aurorix, Manerix) MAOB selective agents: selegiline (Eldepryl, Zelapar, Emsam), rasagiline (Azilect), pargyline (Eutonyl) Harmala alkaloids: harmine, harmaline, tetrahydroharmine, harmalol, harman, norharman *found to varying degrees in Nicotiana tabacum (Tobacco; also cigarettes, cigars, chew, hookah, etc.), Banisteriopsis caapi (Ayahausca, Caapi, Yage), Peganum harmala (Harmal, Syrian Rue), Passiflora incarnata (Passion Flower), and Tribulus terrestris (Puncture Vine), among others*
Indications	*Depression and anxiety, Parkinson's disease (PD) and dementia, for the recreational purpose*
Catechol O-methyl transferase (COMT) inhibitors	Entacapone (Comtan, Stalevo), tolcapone (Tasmar), nitecapone
Indications	*Parkinson's disease (PD)*
DOPA decarboxylase (DDC) inhibitors	Benserazide (Prolopa, Madopar, etc.), carbidopa (Lodosyn, Atamet, Parcopa, Sinemet, Stalevo, etc.), methyldopa (Aldomet, Aldoril, Dopamet, Dopegyt, etc.)
Indications	*Parkinson's disease (PD), sympatholytic or antihypertensive agents*
Dopamine β-hydroxylase (DBH) inhibitors	Disulfiram (Antabuse)
Indications	*Drug addiction as an anticraving agent*
Dopamine β-hydroxylase (DBH) inhibitors	Disulfiram (Antabuse)
Indications	*Drug addiction as an anticraving agent*
Tyrosine hydroxylase (TH) inhibitors	Metirosine (Demser)
Indications	*Pheochromocytoma (PCC) as sympatholytic/antihypertensive agent*
Others	Hyperforin and adhyperforin [Hypericum perforatum (St. John's Wort (SJW))], L-theanine [Camellia sinensis (Tea Plant, also known as Black, White, Oolong, Pu-erh, or Green Tea)], and S-adenosyl-L-methionine (SAMe)
Indications	*Dietary supplements for depression and anxiety*

period coinciding in rodents with the night is the adrenal corticosteroid output that is well known to affect clock genes expression. Interestingly, corticosteroids may also increase noradrenaline uptake in neuroblastoma cells (Sun et al., 2010) and this might happen also in BM cells containing catecholamines.

Circadian variation of the activity of sympathoadrenergic fibers innervating the bone may also affect bone homeostasis. Early studies indeed described increased bone remodeling during light periods in rodents (Simmons and Nichols, 1966). It is now established that β_2-ARs are expressed in osteoblasts and osteoclasts and their stimulation triggers an osteoclastogenic response, while β_1-AR actvation may result in bone protection, and even β_3-ARs may indirectly affect skeleton homeostasis through their effects in other tissues (e.g., the adipose tissue). According to the current hypothesis, increased sympathetic activity could be associated with osteoporosis and the use of β-blockers might result in increased bone mineral density and decreased risk of fractures, although the clinical relevance of such effects is still under scrutiny (reviewed in Elefteriou et al., 2014).

Daily rythmicity of BM catecholamines likely contributes to the circadian control of the immune system, which is now emerging as important regulator of specific immune functions (Scheiermann et al., 2013). In addition, Maestroni et al. (1998) found noradrenaline and dopamine in both short-term and long-term BM cultures as well as in human or murine B lymphoid cell lines, an observation which subsequently prompted thorough investigation of endogenous production of catecholamines by immune cells (Marino et al., 1999). The ability of immune cells to produce and utilize catecholamines likely underlies novel opportunities for the targeted modulation of the immune response: as an example, we described in human CD4+CD25+ regulatory T lymphocytes the occurrence of an autocrine/paracrine loop involving dopaminergic pathways and resulting in down-regulation of their regulatory function (Cosentino et al., 2007), which is apparently involved in autoimmune disease such as multiple sclerosis (Cosentino et al., 2012).

In recent times, interest has risen for dopamine regulating bone marow hematopoiesis. By mans of flow cytometry Spiegel

et al. (2007) showed that human CD34+ cells expressed both DR D_3 and DR D_5 on their surfaces. The more primitive CD34+CD38lo cell populations had higher expression of both DR D_3 and DR D_5 than did the more differentiated CD34+CD38hi cells. Interestingly, dopaminergic agonists increased the polarization and motility of CD34+ cells, as well as their clonogenic progenitor content and engraftment potential. In the same study, by means of flow cytometry, it was shown that human CD34+ cells expressed also the β_2-AR, and G-CSF-mobilized CD34+ cells had higher expression of the β_2-AR than did cord blood CD34+ cells. Adrenaline and noradrenaline regulated CD34+ cell motility and proliferation, *in vitro* as well as *in vivo*, possibily through a canonical Wnt signaling pathway (Spiegel et al., 2007).

Effect of Stress on the Production of Inflammatory Cells

Recently, increasing attention has been dedicated to the mechanisms regulating the trafficking of HSC in the bloodstream. Giudice et al. (2010) reviewed the mechanisms regulating HSC trafficking, showing that circulating HSC exhibit marked circadian fluctuations due to standard cycles of 12 h light/12 h darkness and that circadian HSC oscillations are strongly altered when mice are subjected to continuous light for 2 weeks or to a jet lag. HSC fluctuation is likely in antiphase with the expression of the chemokine CXCL12 in the BM microenvironment. Both circadian HSC trafficking and expression of CXCL12 are modulated by rhythmic release of sympathoadrenergic transmitters in the BM (Giudice et al., 2010). Several lines of evidence indeed suggest that hematopoiesis may be subject to catecholaminergic regulation even under extreme conditions, such as restraint stress and cytostatic treatment (Dygai and Skurikhin, 2011), although also the stress hormone corticosterone may exert major effects on HSC in the BM, as suggested by increased HSC apoptosis and reduced BM repopulation and stromal progenitor cell number following high corticosterone exposure and, on the other side, increased BM HSC and CXCL12 levels in animals with low corticosterone levels or treated with the corticosterone synthesis inhibitor metyrapone (Kollet et al., 2013). Indeed, transcriptome representation analyses showed relative expansion of the selective up-regulation of a subpopulation of immature proinflammatory monocytes (Ly-6c(high) in mice, CD16(-) in humans) within the circulating leukocyte pool in peripheral blood mononuclear cells from people subject to chronic social stress (low socioeconomic status) and mice subject to repeated social defeat (Powell et al., 2013). The effect was ascribed to increased myelopoietic output of Ly-6c(high) monocytes and Ly-6c(intermediate) granulocytes in mice subject to repeated social defeat, and was blocked by treatment with β-AR antagonists as well as with the myelopoietic growth factor GM-CSF. On these basis the authors suggest that sympathoadrenergic-induced up-regulation of myelopoiesis results in a proinflammatory response possibly contributing to the increased risk of inflammation-related disease associated with adverse social conditions (Powell et al., 2013). The ability of chronic stress to induce monocytosis and neutrophilia in humans has been recently reproduced comparing medical ICU residents either off duty or on ICU duty (Heidt et al., 2014), and by use of rodent models it was shown that under conditions of chronic variable stress in mice, sympathetic nerve fibers increase the release of noradrenaline, which in turn signals BM niche cells to decrease CXCL12 levels through β_3-ARs. This leads to increased HSC proliferation and subsequently increased output of neutrophils and inflammatory monocytes (Heidt et al., 2014). Interestingly, treatment of mice with noradrenaline, mimicking acute stress, has been reported to increase circulating levels of CXCL12, resulting in rapid mobilization of HSC, an effect which is induced also by plerixafor (AMD3100), an immunostimulant used to mobilize HSC, and blocked by injection of a β_2-AR antagonist (Dar et al., 2011), suggesting that acute and chronic stress may result in different effects on the BM. The pathological implications of chronic stress-induced monocytosis and neutrophilia were tested in atherosclerosis-prone Apoe(−/−) mice which, when subjected to chronic stress, accelerated hematopoiesis and promoted plaque features associated with vulnerable lesions that cause myocardial infarction and stroke in humans (Heidt et al., 2014). Interestingly, a similar mechanism is likely involved in the expanded neutrophil and monocyte supply which may occur after stroke (Courties et al., 2015). Indeed, in mice with transient middle cerebral artery occlusion (tMCAO), flow cytometry and cell cycle analysis showed activation of the entire hematopoietic tree, including myeloid progenitors resulting in increased expression of myeloid transcription factors, including PU.1, and declined lymphocyte precursors. Notably, In mice after tMCAO, the levels of TH (the first and rate-limiting enzyme in the synthesis of catecholamines) rose in sympathetic fibers and BM noradrenaline levels increased, ass hematopoietic niche factors that promote stem cell quiescence decreased. In mice with genetic deficiency of the β_3-AR, on the contrary, HSCs did not enter the cell cycle in increased numbers after tMCAO (Courties et al., 2015).

Repurposing of Adrenergic and Dopaminergic Agents as Modulators of Hematopoiesis in Health and Disease

The possibility to manipulate hematopoiesis by means of sympathoadrenergic mechanisms provides enormous therapeutic opportunities, also in view of the great amount of adrenergic and dopaminergic agoniststs and antagonistts and indirectly acting agens which are altready in clinical use with a usually favorable therapeutic index (Marino and Cosentino, 2013). Recently, Lucas et al. (2013) provided further cofirmation that that sympathoadrenergic innervation of the BM is crucial for hematopoietic regeneration after chemotherapy. Maestroni et al. (1992) however, who first described *in vivo* the adrenergic modulation of hematopoiesis, showed that chemical sympathectomy by 6-hydroxydopamine (6-OHDA) increased peripheral blood leukocytes after syngeneic BM transplantation in mice. Lucas et al. (2013), on the contrary, reported reduced survival in 6-OHDA-treated animals, and differences are hardly explained by experimental conditions, as both mice strain and gender, as well as chemical denervation

protocol, are the same (except for additional 250 mg/kg of 6-OHDA on day 2 after the initial 100 mg/kg on day 0). In addition, in the study by Maestroni et al. (1992) differences between saline- ad 6-OHDA-treated animals were evident only in animals kept under continuous 24-h lighting, and not in those kept under a 12:12 light:dark cycle. Finally, in the study by Maestroni et al. (1992) the effect of sympathetic denervation was concentration-dependently mimicked by the α_1-AR antagonist prazosin, while the non selective β-AR antagonist propranolol was without effect *per se* and selectively reverted the effect of prazosin on platelets. Lucas et al. (2013) used only β_2- and β_3-AR antagonists, and only at one dose, which resulted in mild effects qualitatively similar to those of 6-OHDA. It is possible that differences in the effects of 6-OHDA on BM and circulating cell recovery may depend upon the different doses used. In the article by Lucas et al. (2013) we found no information concerning the actual effects of 6-OHDA on TH+ nerve endings in BM or on blood cells. Indeed, 6-OHDA can exert direct toxicity on circulating lymphocytes (Del Rio and Velez-Pardo, 2002), and high doses might be therefore less selective for nerve endings. Anyway, clarifying such minor methodological and procedural issues will pave the way for clinical trial of adrenergic agents as promoters of hematopoietic recovery.

Evidence obtained in rodents indicate that β_2-AR agonists may enhance mobilization of HSC and hematopoietic progenitor cells. Katayama et al. (2006) showed that after administration of the β_2-AR agonist clenbuterol, the mobilization defect was partly rescued in $Dbh^{-/-}$ mice (lacking dopamine β-hydroxylase, the enzyme which converts dopamine into noradrenaline) and resulted in enhanced mobilization in $Dbh^{+/-}$ animals. Clenbuterol was effective only before and during G-CSF administration. The authors propose that the effect of G-CSF is due to release of noradrenaline from sympathetic nerve endings resulting in osteoblast suppression and reduced synthesis of CXCL12, through the activation of β_2-ARs which cooperate with other signals from the G-CSF receptor (Katayama et al., 2006).

Sympathoadrenergic agents may also contribute to restore normal hematopoiesisis in myeloproliferative neoplasms. Sympathoadrenergic fibers, supporting Schwann cells and nestin(+) mesenchymal stem cells are reduced in the BM of patients with myeloproliferative neoplasms as well as in mice expressing the human JAK2(V617F) mutation in HSCs. Interestingly, reduction of mesenchymal stem cells is due to BM neural damage and Schwann cell death triggered by IL-1β, resulting in expanded mutant HSC number and accelerated myeloproliferative neoplasms progression. Treatment with β_3-AR agonists restore the sympathetic regulation of nestin(+) mesenchymal stem cells, blocking myeloproliferative neoplasms progression by indirectly reducing the number of leukaemic stem cells (Arranz et al., 2014).

Neuropathy of sympathoadrenergic fibers has been recently proposed also as a novel mechanism for malignancies like acute myelogenous leukemia (AML) to exploit the hematopoietic microenvironment for its purposes (Hanoun et al., 2014). Indeed, in an animal model of AML, of sympathetic nervous system neuropathy promotes leukemic BM infiltration, possibly through an expansion of perivascular mesenchymal stem and progenitor cells primed for osteoblastic differentiation at the expense of the physiological periarteriolar niche cells. Blockade of β_2-AR pathways enhanced AML infiltration whereas a β_2-AR agonist reduced disease activity.

As a final remark, we would like to mention the recently emerging evidence which indicate the multiple ways in which the local microenvironment may contribute to cancer-induced bone disease, possibly through a key role of the sympathetic nervous system providing bone homeostatic signals. Stress and anxiety are able to cause bone loss through the sympathetic nervous system, and have been shown to have an effect on not only the osteolytic effect of breast cancer, but also the metastatic infiltration of bone. Sympathetic nervous system signaling to β-ARs on osteoblasts has also been implicated in potentiating other signals, such as parathyroid hormone, osteopontin and IGF-1, and release of HSCs from their niche, which may also have implications for invading cancers. Preliminary evidence have been recently summarized into an excellent review (Olechnowicz and Edwards, 2014) and the topic will likely attract the broadest interest in the near future.

Conclusion

Although the first *in vivo* evidence for the role of sympathoadrenergic fibers in the modulation of hematopoiesis was provided less than 25 years ago (Maestroni et al., 1992), the relevance of catecholaminergic modulation of hematopoiesis rapidly raised thanks to several seminal studies showing the key role of sympathoadrenergic fibers in the hematopoietic niche, as well as the potential of adreneceptor ligands, and in some cases even of dopamine receptor ligands (Sarkar et al., 2014).

In addition, the recently established notion that activation of sympathoadrenergic represents a link between chronic stress (e.g., due to adverse social conditions) and up-regulation of proinflammatory responses, such as monocytosis and neutrophilia in humans (see e.g., Heidt et al., 2014), not only provides a mechanistic explanation to the negative prognostic role of the neutrophil/lymphocyte ratio in a broad and heterogeneous number of critical conditions, such as cancer (Templeton et al., 2014) and cardiovascular disease (Guasti et al., 2011; Bhat et al., 2013) but also offers several opportunities for therapeutic intervention. Results obtained so far in preclinical models would already support to various extent the clinical evaluation of: the α_1-AR antagonist prazosin (Maestroni et al., 1992; Maestroni and Conti, 1994), β_2-AR agonists (Katayama et al., 2006; Dar et al., 2011) and dopaminergic agonists (Spiegel et al., 2007) for HSC transplantation; α_1-AR agonists (Togni and Maestroni, 1996; Maestroni et al., 1997) as well as dopaminergic agonists (Sarkar et al., 2014) to protect against the adverse effects of cytotoxic agents on BM; β-AR antagonists to reduce the proinflammatory response associated with chronic stress (Powell et al., 2013; Heidt et al., 2014); β_2-AR agonists (Hanoun et al., 2014)

and β_3-AR agonists (Arranz et al., 2014) in myeloproliferative disease.

Sympathoadrenergic innervation has finally reached an established role in the complex network of neural and neuroendocrine agents which regulate the hematopoietic system (Maestroni, 2000). Several key questions still await answers, including whether the neural regulation of hematopoiesis plays any role in aplastic anemia, leukemia, and immune-based diseases or during emergencies such as acute infections and/or stress events: any positive response will provide the conceptual framework for the straightforward development of new pharmacological strategies, considering the availability of several dopaminergic and adrenergic agents, already in clinical use for non-immune indications and with a usually favorable risk-benefit profile. Finally, from a general point of view, these findings include hematology among the fields which cannot but benefit from an integrative neuroimmune pharmacological approach (Izeku and Gendelman, 2008).

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frontiers in Cellular Neuroscience

MINI REVIEW
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Impact of electromagnetic fields on stem cells: common mechanisms at the crossroad between adult neurogenesis and osteogenesis

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In the recent years adult neural and mesenchymal stem cells have been intensively investigated as effective resources for repair therapies. *In vivo* and *in vitro* studies have provided insights on the molecular mechanisms underlying the neurogenic and osteogenic processes in adulthood. This knowledge appears fundamental for the development of targeted strategies to manipulate stem cells. Here we review recent literature dealing with the effects of electromagnetic fields on stem cell biology that lends support to their use as a promising tool to positively influence the different steps of neurogenic and osteogenic processes. We will focus on recent studies revealing that extremely-low frequency electromagnetic fields enhance adult hippocampal neurogenesis by inducing epigenetic modifications on the regulatory sequences of genes responsible for neural stem cell proliferation and neuronal differentiation. In light of the emerging critical role played by chromatin modifications in maintaining the stemness as well as in regulating stem cell differentiation, we will also attempt to exploit epigenetic changes that can represent common targets for electromagnetic field effects on neurogenic and osteogenic processes.

Keywords: epigenetics, extremely-low frequency electromagnetic fields, gene expression programs, mesenchymal stem cells, neural stem cells

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Introduction

Any adult tissue with repair/regenerative capabilities contains tissue-specific stem cells (SCs) defined as clonogenic, self-renewing cells that retain proliferative and differentiation potential allowing to preserve tissue homeostasis and to repair injury (Anderson et al., 2001). Unlike differentiated cells, adult SCs are unspecialized cells that can self-renew to replenish themselves and differentiate into one or more specialized cell types within a committed lineage (Minguell et al., 2001). As such SCs hold promise for tissue/organ repair with the ultimate goal to regenerate and restore normal functions. Adult SCs are most often in a quiescent state, and either or both intrinsic or extrinsic factors can trigger programs for self-renewal or differentiation (Kobilo et al., 2011; Podda et al., 2013; Leone et al., 2014). It is currently accepted that a combination of niche signals and cell intrinsic programs orchestrate the transition from an undifferentiated stem cell state to a progenitor cell committed to the final fate. Among multiple sources of adult stem cells, neural SCs (NSCs) and mesenchymal SCs (MSCs) have been intensively studied for their role in brain and bone physiology as well as for their potential use in

cell-based therapies for treating neurological/neurodegenerative diseases and for reconstructive surgery, respectively (Yamaguchi, 2014; Hayrapetyan et al., 2015; Lin and Iacovitti, 2015).

In this context, great effort has been put to identify stimuli and molecular pathways influencing the neurogenic and osteogenic processes. Within this scenario here we review recent literature focusing on epigenetic mechanisms that appear to be crucially involved in the process of both neurogenesis and osteogenesis. We will also discuss the involvement of chromatin modifications in mediating the effects of extremely-low frequency electromagnetic field (ELFEF) stimulation that is emerging as an effective tool to positively modulate neurogenic and osteogenic processes.

Neural Stem Cells

In the adult mammalian brain, NSCs reside mainly in two discrete regions: the subgranular zone of the hippocampal dentate gyrus and the subventricular zone of the lateral ventricles (Gage, 2000; Ming and Song, 2011). Throughout life these neurogenic niches ensure continuous production of new neurons and maintain the NSC pool (Kempermann et al., 2004). NSC self-renewal is intrinsically sustained by specific "stemness" genes, including those controlled by Notch signaling (Louvi and Artavanis-Tsakonas, 2006; Ables et al., 2010). NSC differentiation results from the gradual inactivation of "stemness" genes and the activation of pro-neural genes including, *Ascl1* (Achaete-Scute Complex-Like 1, also known as *Mash1*), *Neurogenin1* and *NeuroD1*.

Recent studies have also revealed a key role of Wnt/β-catenin signaling in balancing NSC self-renewal and neuronal differentiation. In particular, NeuroD1 has been reported to be the downstream mediator of Wnt pathway and its expression is silenced in undifferentiated NSCs. In the presence of extracellular Wnt, β-catenin accumulates in the nucleus, resulting in NeuroD1 activation and subsequent neuronal differentiation (Kuwabara et al., 2009). A similar molecular mechanism has been described for the transcription factor cAMP response element-binding protein (CREB), which modulates neuronal differentiation by binding regulatory sequences of pro-neural genes (Deisseroth et al., 2004; Jagasia et al., 2009). In particular, Ca^{2+} signaling triggers phosphorylation of CREB, that, once activated, promotes NSC differentiation (West et al., 2001; Giachino et al., 2005; D'Ascenzo et al., 2006; Leone et al., 2014).

Mesenchymal Stem Cells (MSCs)

MSCs are generally derived from the bone marrow (Friedenstein et al., 1987; Pittenger et al., 1999; Lin et al., 2013), but they can also be sourced from other tissues including umbilical cord blood and adipose tissue. MSCs give rise to mesenchymal phenotypes including bone, cartilage and fat, and to non-mesenchymal cells including neural cells. Numerous studies, primarily focusing on bone cell lineages, have been performed to get insight into MSC differentiation process (Minguell et al., 2001; Fakhry et al., 2013).

Bone formation is regulated by osteogenic transcription factors that mediate the staged expression of bone phenotypic genes, such as the osteocalcin (OC) gene, during differentiation of osteoprogenitor cells to mature osteoblasts. In particular, signaling molecules such as bone morphogenetic proteins (BMPs) and Wnt pathway favor osteoblastogenesis, while Notch1 and its downstream target Hes1 inhibit osteoblast differentiation. Recently, it has been shown that the transcriptional factor Runx2, a major target of BMP pathway, induces osteoblast differentiation by repressing *Hes1* and by activating *OC* and other bone-related genes (Ann et al., 2011; Wang et al., 2013).

Epigenetic Mechanisms in Neurogenesis and Osteogenesis

Increasing body of evidence supports the view that epigenetic mechanisms including DNA methylation and histone modifications orchestrate SC self-renewal, lineage commitment, cell fate specification and terminal differentiation. These regulatory mechanisms promote the formation of relatively "open" and "poised" epigenetic states that, by regulating transcriptional activity, mediate the execution of lineage-specific gene expression programs.

Consistent with this concept, transcriptional control of both adult neurogenesis and osteogenesis is under intensive regulation by epigenetic modifications of the regulatory sequences of pro-neural genes including *Ascl1*, *Neurogenin1* and *NeuroD1* (Ma et al., 2010; Hsieh, 2012; Eslaminejad et al., 2013; Amador-Arjona et al., 2015) and bone-related genes such as *OC* (Gutierrez et al., 2002; Eslaminejad et al., 2013), respectively.

DNA Methylation

DNA methylation refers to addition of methyl group to the carbon 5 position of the DNA base cysteine, which results in the generation of 5-methylcytosine (5-mC). DNA methylation is catalyzed by DNA-methyl-transferase (DNMT) and usually results in gene repression. DNMT3a and DNMT3b establish *de novo* methylation, whereas DNMT1 maintains methylation patterns during cell division. *De novo* methylation and maintenance of methylation marks, either directly or indirectly affecting gene expression, are capable of regulating sequential steps of adult neurogenesis (Covic et al., 2010; Hsieh and Eisch, 2010).

Seemingly, DNA methylation is dynamically involved in MSC bone differentiation. A significant hypermethylation at the *OC* locus has been associated with its repression. Accordingly, during osteoblast differentiation this CpG methylation significantly decreased, resulting in enhanced *OC* expression (Villagra et al., 2006). Furthermore, Dansranjavin et al. (2009) demonstrated that MSC differentiation into osteoblast cells was accompanied by reduced expression of the stemness genes via hypermethylation of their promoters.

Histone Modifications

Gene expression also depends on DNA accessibility, which is determined by histone post-transcriptional modifications, such as acetylation and methylation that commonly activate and repress gene expression, respectively. These modifications

have been involved in both adult neurogenesis and osteogenesis (Hsieh and Eisch, 2010; Ma et al., 2010). Histone acetylation is a dynamic process regulated by both histone acetyltransferases (HATs) and deacetylases (HDACs) that add or remove acetylation marks, respectively. Transcriptional repression through HDAC activity is essential for adult NSC proliferation and self-renewal. For example, the expression of the Notch effector, Hes1, regulates NSC self-renewal by interacting with different HDACs to repress pro-neural gene expression (Hsieh et al., 2004; Kuwabara et al., 2009; Sun et al., 2011; Zhou et al., 2011). On the other hand, enhanced adult NSC differentiation has been associated with increased H3 acetylation levels and the expression of CREB-binding protein (CBP), a critical histone acetyltransferase (HAT) for neuronal differentiation (Chatterjee et al., 2013). Thus, maintenance of histone acetylation appears important for neuronal lineage progression of adult NSCs, while histone deacetylation seems relevant for NSC self-renewal.

Histone acetylation/deacetylation has also been involved in osteogenesis. Acetylation of histone H4 and to a lesser extent, of H3 at the *OC* promoter accompanies the induction of *OC* expression in mature osteoblasts (Shen et al., 2003). Accordingly, the down-regulation of HDAC1 is associated to osteoblast differentiation (Lee et al., 2006).

Adult neurogenesis and osteogenesis are also under tight epigenetic control of histone methylation that is regulated by two antagonistic complexes: (i) Polycomb (PcG), that promotes H3 lysine 27 tri-methylation (H3K27me3); and (ii) Trithorax (TrxG), that promotes H3 lysine 4 tri-methylation (H3K4me3).

In NSCs, depletion of PcG components, such as Ezh2, largely removed H3K27me3 markers, de-repressed a wide panel of genes, and delicately altered the balance between self-renewal and differentiation as well as the timing of neurogenesis (Hsieh and Eisch, 2010; Pereira et al., 2010).

Osteogenic lineage determination has been associated to chromatin hyperacetylation and H3K4 hypermethylation of different genes, including *OC* (Hassan et al., 2007; Wei et al., 2011).

The literature reviewed above highlights a prominent role of epigenetic mechanisms in the modulation of gene expression during neurogenesis and osteogenesis processes. Interestingly, experimental evidence involved these mechanisms in the beneficial effects of ELFEF stimulation on adult hippocampal neurogenesis (**Figure 1**).

Effects of Electromagnetic Fields on Neural and Mesenchymal Stem Cells

It is widely reported that electromagnetic fields modulate different steps of neurogenesis and osteogenesis and several potential cellular targets have been identified. However, the heterogeneity of exposure systems and experimental protocols chosen has produced a complex picture in which data may appear at first sight inconsistent. On the other hand, when comparing data obtained under similar exposure conditions then they appear more homogeneous (Di Lazzaro et al., 2013). From this perspective here we focused on ELFEFs and documented that such stimulation effectively promotes proliferation and functional differentiation of both NSCs and MSCs, likely engaging similar molecular pathways.

With regard to NSCs, our initial studies showed that ELFEF stimulation (1 mT, 50 Hz) enhanced differentiation of adult cortical NSCs (Piacentini et al., 2008). In line with what reported in other cell models (Grassi et al., 2004a; Wolf et al., 2005), ELFEF stimulation increased proliferation of undifferentiated NSCs but, once the differentiation process had started, ELFEFs inhibited proliferation and increased the percentage of cells acquiring molecular markers and functional properties of neurons. Molecular and electrophysiological data showed that these effects were linked to enhanced expression and function of voltage-gated L-type calcium channels (Ca_v1) (Grassi et al., 2004b; D'Ascenzo et al., 2006; Piacentini et al., 2008). These findings prompted subsequent studies (Cuccurazzu et al., 2010) aimed at investigating the effects of ELFEFs on the expression of genes regulating NSC fate given the well-recognized prominent role played by intracellular Ca^{2+} signaling in such mechanisms (West et al., 2001; Deisseroth et al., 2004). In particular, i*n vivo* and *in vitro* studies on adult hippocampal neurogenesis demonstrated that ELFEF-induced Ca^{2+} influx through Ca_v1 channels led to increased CREB phosphorylation and that was a crucial step in regulating the expression of genes responsible for NSC proliferation and neuronal differentiation (Cuccurazzu et al., 2010). Indeed, quantitative RT-PCR analysis of hippocampal extracts from adult mice exposed to ELFEFs (50 Hz, 1 mT; 7 h/day for 7 days) revealed increased transcription of *Ascl1*, *NeuroD2*, and *Hes1* paralleled by higher levels of mRNA encoding α_{1C} and α_{1D} subunits of $Ca_v1.2$ and $Ca_v1.3$ channels. Enhanced expression of NeuroD1, NeuroD2, and the Ca_v1 channel proteins in the hippocampi of ELFEF-exposed mice was also confirmed by Western blot analysis. Immunofluorescence analyses revealed that *in vivo* ELFEF stimulation affected NSC proliferation and neuronal differentiation, as shown by increased numbers of cells labeled for the proliferation marker 5-bromo-2′-deoxyuridine (BrdU), and double-labeled for BrdU and the immature neuronal marker doublecortin. Interestingly, 30 days after the end of the ELFEF stimulation protocol ∼50% of the newborn neurons became mature granule cells that were functionally integrated in the dentate gyrus network, as demonstrated by neurophysiological indexes. In particular, in hippocampal brain slices from ELFEF exposed mice, long-term potentiation at medial perforant path-dentate granule cell synapses in the presence and in the absence of $GABA_A$ receptor blockade was significantly greater than that observed in unexposed control mice (Cuccurazzu et al., 2010), as expected as a consequence of enhanced number of newborn neurons integrated in the local circuit (Massa et al., 2011).

In a subsequent study we demonstrated that *in vivo* ELFEF stimulation also promoted the survival of hippocampal newly generated neuron by rescuing them from apoptotic cell death, an effect associated with enhanced expression of pro-survival protein Bcl-2 and decreased expression of the apoptotic protein Bax (Podda et al., 2014).

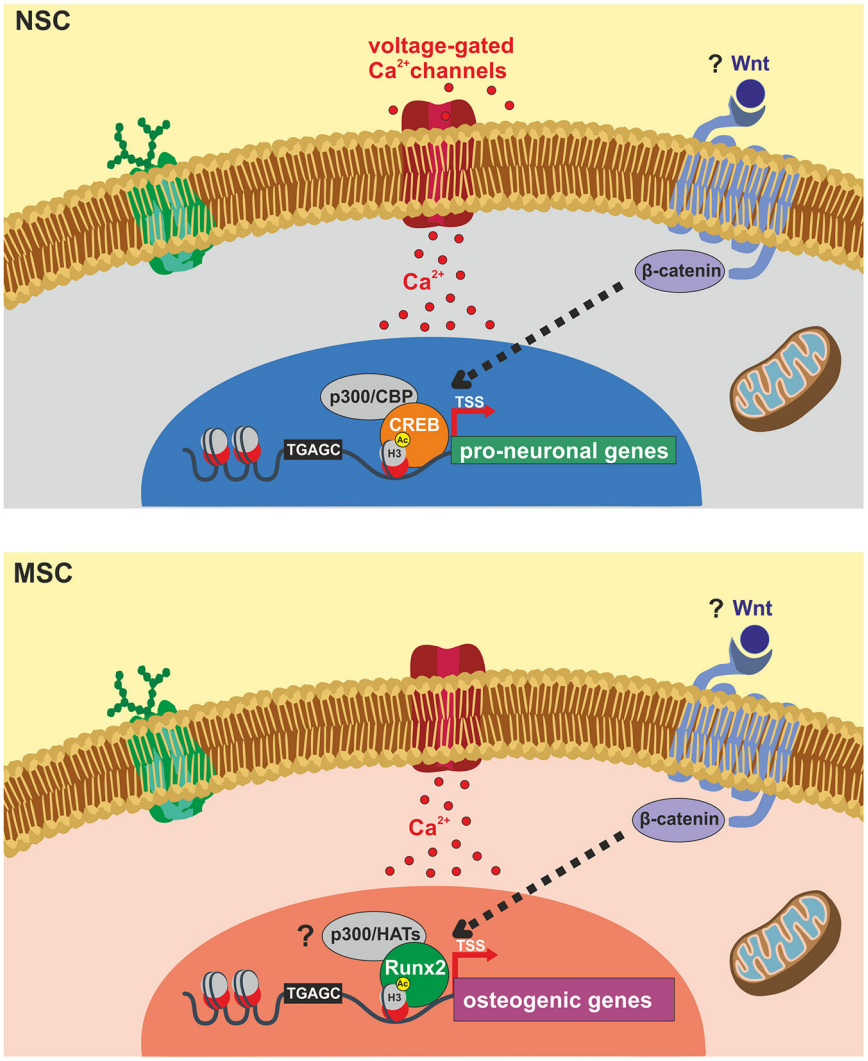

FIGURE 1 | Schematic representation of identified molecular targets involved in ELFEF-induced enhancement of adult hippocampal neurogenesis and osteogenesis. Experimental evidence demonstrates that ELFEFs affect key molecular players involved in the up-regulation of pro-neuronal genes in hippocampal neural stem cells (NSCs, upper panel) and bone-related genes in mesenchymal stem cells (MSCs, lower panel). In hippocampal NSCs ELFEFs enhance pro-neuronal gene expression by a mechanism involving: (i) Ca^{2+}-dependent phosphorylation/activation of CREB and its binding on pro-neuronal gene promoters; (ii) increased recruitment of the HAT CREB-binding protein (CBP) on the same regulatory sequences; (iii) enhanced histone 3 (H3) acetylation on lysine 9 (H3Ac) of pro-neuronal gene promoters (i.e., *NeuroD1* and *Neurogenin1*). In MSCs ELFEF-induced up-regulation of bone-related genes has been also linked to intracellular Ca^{2+} signaling and enhanced expression of the transcription factor Runx2 which is known to bind the regulatory sequences of osteogenic genes promoting their expression. Question marks indicate putative common molecular targets of ELFEFs in NSCs and MSCs including: (i) Wnt/β-catenin signaling regulating pro-neuronal and osteogenic gene expression at transcriptional and epigenetic levels; (ii) activation of p300 or other HATs by Runx2, resembling the pCREB/CBP pathway activated by ELFEFs in hippocampal NSCs.

Importantly, our most recent study demonstrated that the ELFEF-induced enhancement of hippocampal neurogenesis and synaptic plasticity lead to improved hippocampal-dependent learning and memory in mice (Leone et al., 2014). This study shed further light on molecular mechanisms underlying ELFEFs' effects revealing a significant regulation of epigenetic mechanisms leading to pro-neuronal gene expression. In particular, in *in vitro* and *in vivo* models of adult hippocampal neurogenesis we demonstrated that enhanced expression of *Hes1* in proliferating NSCs and *NeuroD1*, and *Neurogenin1* in differentiating NSCs were associated to increased H3K9 acetylation and Ca^{2+}-dependent CREB/CBP recruitment on the regulatory sequence of these genes (Leone et al., 2014). This study suggested that regulation of epigenetic mechanism provides a fine and targeted control of neurogenic process by ELFEFs.

Concerning MSCs, it is worth noting that, although the neuronal transdifferentiation of somatic SCs for reparative strategies in neurodegenerative diseases is still debated (Lu et al., 2004), several studies reported the effects of 50 Hz ELFEFs in promoting neuronal differentiation of MSCs from various

sources including bone marrow, supporting a strong effects of this stimulation on pro-neurogenic pathways.

The work by Cho et al. (2012) showed that ELFEFs (50 Hz, 1 mT for 12 days) increased neuronal differentiation of human bone marrow-derived (hBM)-MSCs, inducing the expression of neural cell markers including NeuroD1. Similar results were obtained by Bai et al. (2013) using similar ELFEF parameters (50 Hz, 5 mT for 12 days). More recently, Seong et al. (2014) showed that ELFEF exposure (50 Hz, 1 mT for 8 days) of hBM-MSCs promoted neuronal differentiation even in the absence of any neurotrophic factor. Indeed, exposed hBM–MSCs showed significant increase of NeuroD1 expression as well as electrophysiological properties of neurons. The same authors demonstrated that ELFEFs enhanced differentiation of mouse NSCs towards the neuronal phenotype. Analysis of the transcriptome of ELFEF-exposed hBM-MSCs and mouse NSCs revealed dramatic changes in global gene expression in both cell types compared to unexposed cells, with relevant up-regulation of several transcription factors, such as Egr1, DNA-binding protein inhibitor ID-1 and Hes1. In particular, Egr1, regarded as a strong early neurogenic transcription factor, appeared to be the most highly upregulated in neuronal differenting cultures from hBM-MSCs and mouse NSCs. Seong et al. (2014) further confirmed the role of Egr1 in mediating the pro-neurogenic effect of ELFEFs on MSCs showing that: (i) knockdown of Egr1 in the hBM–MSCs significantly inhibited ELFEF induced neuronal differentiation; (ii) the overexpression of Egr1 combined with ELFEF exposure increased the efficiency of cell-replacement therapy thus alleviating neurological symptoms in a Parkinson's disease mouse model.

Besides the key finding of the study involving the transcription factor Egr1 in ELFEF effects, it is interesting to note that the list of genes modulated by ELFEFs includes HDACs (i.e., HDAC5 and HDAC11) that are known to critically regulate SC self-renewal and differentiation (Cheng et al., 2005; Sun et al., 2011; Zhou et al., 2011). Unfortunately, the study by Seong and co-workers did not specifically address the issue of whether histone modifications were involved in ELFEF mediated up-regulation of neuronal genes.

Besides the studies exploring the potential to promote neuronal transdifferentiation of MSCs, ELFEFs have been well known for many years as potent stimuli to promote ostegenesis and cartilage formation (Heckman et al., 1981). In this respect the majority of studies were performed by using pulsed EFs (PEMF, frequencies in the range of 7.5–75 Hz) and, given their efficacy, devices producing such stimuli are currently approved by the US Food and Drug Administration for the treatment of fracture non-unions and osseous defects (Assiotis et al., 2012; Boyette and Herrera-Soto, 2012). Initially, clinical effectiveness of EFs was attributed to the accelerated formation of bone matrix by the weak electric current generated by the magnetic field (de Haas et al., 1980; Aaron and Ciombor, 1996). However, more recent studies have clearly involved MSCs as target of EF action.

Indeed, studies performed on bone marrow-derived stromal cells (BMSCs) demonstrated that exposure to PEMF stimulates cell proliferation as well as osteogenesis by increasing early osteogenic markers including Runx2/Cbfa1 and alkaline phosphatase (ALP; Pittenger et al., 1999; Sun et al., 2009; Tsai et al., 2009).

The effects of PEMFs on osteogenic differentiation of adipose-derived stem cells (ASCs) have been more recently investigated. In particular, PEMF treatment enhanced the expression of bone matrix genes (OC and collagen type I in ASC) as well as bone mineralization (Ceccarelli et al., 2013; Chen et al., 2013; Ongaro et al., 2014). Additionally, recent lines of evidence suggest that sinusoidal ELFEF stimulation promotes proliferation and osteogenic differentiation of both BMSCs (Zhong et al., 2012) and ASCs (Kang et al., 2013).

At present the mechanism by which PEMFs/ELFEFs promote the formation of bone remains elusive and future studies are highly demanded. Some evidences indicate that, as documented for NSCs, the electromagnetic stimulation raises the net Ca^{2+} flux and expression/activation of Ca^{2+}-binding proteins such as calmodulin in human osteoblast-like cells and MSCs (Fitzsimmons et al., 1994; Lim et al., 2013). The increase in the cytosolic Ca^{2+} concentration is the starting point for signaling pathways targeting specific bone matrix genes and, in keeping with this, the application of the electromagnetic waves was shown to increase the level of transcripts of osteogenesis-related genes including those encoding for decorin, osteopontin, collagen type-I and Runx2 (**Figure 1**).

Conclusions

The recent findings in stem cell biology have opened a new window in the expanding area of regenerative medicine based on tissue engineering and cell therapy derived from a variety of SCs, including NSCs and MSCs.

With regard to neurogenesis and ostegenesis it is becoming increasingly clear that these processes rely on the activation of specific and complex transcriptional programs whose regulation may provide a cellular candidate for therapeutic intervention. In this context epigenetic mechanisms play a critical regulatory role translating a wide array of endogenous and exogenous signals into persistent changes in gene expression in both NSCs and MSCs. ELFEF stimulation has been recognized as effective tool in promoting both neurogenesis and osteogenesis and studies performed so far on NSCs point to chromatin remodeling as a critical determinant in ELFEF's induced pro-neuronal gene expression. The literature here reviewed suggests that epigenetic regulation of bone-related gene may seemingly mediate the effects exerted by EFs on osteogenesis.

It is our opinion that future research on different types of SCs may benefit from higher degree of communication between the different fields that would contribute to uncover more than expected common molecular pathways and stimulation paradigms of potential relevance for therapeutic interventions.

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frontiers in Cellular Neuroscience

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Meninges harbor cells expressing neural precursor markers during development and adulthood

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Brain and skull developments are tightly synchronized, allowing the cranial bones to dynamically adapt to the brain shape. At the brain-skull interface, meninges produce the trophic signals necessary for normal corticogenesis and bone development. Meninges harbor different cell populations, including cells forming the endosteum of the cranial vault. Recently, we and other groups have described the presence in meninges of a cell population endowed with neural differentiation potential *in vitro* and, after transplantation, *in vivo*. However, whether meninges may be a niche for neural progenitor cells during embryonic development and in adulthood remains to be determined. In this work we provide the first description of the distribution of neural precursor markers in rat meninges during development up to adulthood. We conclude that meninges share common properties with the classical neural stem cell niche, as they: (*i*) are a highly proliferating tissue; (*ii*) host cells expressing neural precursor markers such as nestin, vimentin, Sox2 and doublecortin; and (*iii*) are enriched in extracellular matrix components (e.g., fractones) known to bind and concentrate growth factors. This study underlines the importance of meninges as a potential niche for endogenous precursor cells during development and in adulthood.

Keywords: meninges, neural precursor cells, fractones, nestin, brain development, proliferation, neural stem cell niche

Introduction

Over the last years, new and unexpected roles for meninges have emerged (Decimo et al., 2012a; Richtsmeier and Flaherty, 2013; Bjornsson et al., 2015). Not just a protective fluid-filled membranous sac enclosing the brain, meninges form a complex microenvironment endowed with soluble trophic factors, extracellular matrices and cells playing fundamental roles in both skull and brain development (Richtsmeier and Flaherty, 2013; Bjornsson et al., 2015). In the developing rat, meninges begin to form at embryonic day 11 (E11) appearing as an undifferentiated mesenchymal network of cells located between the epidermis and the brain (Angelov and Vasilev, 1989). The bones of the skull start to form by E14, whereas meninges complete their differentiation and appear as a three-layered tissue (the outer dura mater, the inner pia mater and the intermediate arachnoid) only after E19 (Mercier and Hatton, 2000; Mercier et al., 2002; Kokovay et al., 2008; Bjornsson et al., 2015). At the end of brain development, meninges penetration and distribution inside the

central nervous system (CNS) parenchyma is abundant and complex (Mercier and Hatton, 2000; Mercier et al., 2002). Indeed, extroflexions of the pia and arachnoid membranes (leptomeninges) form a perivascular space (Virchow–Robin space) around every vessel of the CNS (Reina-De La Torre et al., 1998; Rodriguez-Baeza et al., 1998).

Meninges-derived extracellular matrix components (e.g., laminin, heparan sulfate proteoglycans, collagen IV and fibronectin) have been shown to be essential for the correct development of the cortex (Halfter et al., 2002; Beggs et al., 2003). In addition, several molecules playing critical functions in cranial bone and brain development and homeostasis have been shown to be produced by meninges (Radakovits et al., 2009; Richtsmeier and Flaherty, 2013); these include fibroblast growth factors (FGFs) (Mercier and Hatton, 2001), insulin-like growth factor-II (Stylianopoulou et al., 1988), retinoic acid (RA) (Siegenthaler et al., 2009), stromal cell-derived factor-1 (SDF-1, also referred to as CXCL12) (Borrell and Marin, 2006; Belmadani et al., 2015) and transforming growth factor beta family proteins (Choe et al., 2014).

Meningeal cells of the dura mater may function as endosteum of the cranial vault (Adeeb et al., 2012; Richtsmeier and Flaherty, 2013). Moreover, we have recently found that leptomeninges of adult rodent brain and spinal cord host a population of cells expressing the neural precursor markers nestin and doublecortin (DCX) (Bifari et al., 2009; Decimo et al., 2011). A similar cell population was also described in human meninges (Decimo et al., 2011; Petricevic et al., 2011). Cells isolated from both brain and spinal cord leptomeninges could be differentiated into neurons and oligodendrocytes *in vitro*; after transplantation *in vivo* these cells integrate in hippocampal CA1 region acquiring neuronal morphology (Bifari et al., 2009). Of note, following injury meningeal cells increase their proliferation rate, migrate into the parenchyma, contribute to the injury-induced reaction (Decimo et al., 2011; Kumar et al., 2014) and increase their expression of neural progenitor markers (Decimo et al., 2011; Nakagomi et al., 2011, 2012; Ninomiya et al., 2013).

Interestingly, this pattern of reactivity to injury (increased proliferation, expression of progenitor markers and migration) is a typical feature of the well-described neural stem cell niche of the subventricular zone (SVZ) (Decimo et al., 2012b; Bjornsson et al., 2015). Here, the niche shows a peculiar microenvironment that provides conditions for maintenance of the stem cell pools in a quiescent state as well as signals for activation and differentiation when neurogenesis is required (Scadden, 2006; Decimo et al., 2012a,b; Bjornsson et al., 2015).

Considering the fundamental role of meningeal cells during brain development, the presence of cells expressing markers of stemness and their activation following CNS injury, we asked whether leptomeninges share some of the features of a neural stem cell niche. To this aim we analyzed by morphological, molecular and biochemical criteria: (i) the number and the proliferation rate of leptomeningeal cells; (ii) the presence and the distribution of cells expressing neural progenitor markers; and (iii) the distribution of some of the known extracellular components of neural niches. Since the primary feature of a stem cell niche is the capability to harbor and maintain precursors, in this study we analyzed rat brain leptomeninges in embryo, at birth, during weaning and in adult animals.

Materials and Methods

Tissue Preparation for Immunofluorescence

Animal housing and all the protocols involving the use of experimental animals in this study were carried out in accordance with the recommendations of the Italian Ministry of Health (approved protocol N. 154/2014-B). Sprague-Dawley (SD) rats at different developmental stages (embryonic day 14: E14; embryonic day 20: E20; at birth: P0; after weaning at postnatal day 15: P15; young adult at 6–8 weeks and mature adult at 24 weeks) were anesthetized by intraperitoneal injection with chloral hydrate (350 mg/kg) and sacrificed by intracardial perfusion of PBS with 4% paraformaldehyde (PFA)/4% sucrose (pH 7.4) solution. Brains were extracted, fixed in 4% PFA solution and transferred into 10% and subsequently 30% sucrose solution. By cryostat cutting, 40 μm thick coronal brain sections were obtained and processed by immunofluorescence.

Immunofluorescence and Quantitative Analysis

Brain slices were incubated for 2 h in blocking solution (5%FBS/3%BSA/0.3% Triton X-100 in PBS) and then incubated overnight at 4°C with primary antibodies. Primary antibodies were detected with appropriate secondary antibodies for 4 h at 4°C in blocking solution. Slices were incubated for 10 min with the nuclear dye TO-PRO 3 (Invitrogen). Staining for the nuclear marker of proliferation Ki67 required antigen retrieval prior to the standard protocol applied in this study; slides were therefore incubated for 30 s in citrate buffer (10 mM trisodium citrate dihydrate/0.05% Tween-20 pH 6.0). Quantification of Ki67-, nestin-, vimentin-, Sox2-, and DCX-positive cells and nuclei was done by counting positive cells above the basal lamina (identified by laminin reactivity) in at least 18 sections for each time point ($n \geq 3$ animals analyzed). Acquisition parameter settings (pinhole, gain, offset, laser intensity) were kept fixed for each channel in different sessions of observation at the confocal microscope.

Antibodies

The following primary antibodies were used: anti-nestin (mouse, 1:1000, BD Pharmingen), anti-laminin (rabbit, 1:1000, Sigma), anti-Ki67 (rabbit, 1:100, Abcam), anti-vimentin (chicken, 1:1000, Millipore), anti-Sox2 (goat, 1:200, Santa Cruz), anti-DCX (goat, 1:100, Santa Cruz), anti-Tuj1 (mouse, 1:1000, Covance) and anti-heparan sulfate (mouse, 1:500, US Biological).

The following secondary antibodies were used: goat anti-mouse CY3 (Amersham), donkey anti-mouse 488 (Molecular Probes), goat anti-rabbit 488 (Molecular Probes), donkey anti-rabbit 488 (Molecular Probes), rabbit anti-chicken CY3 (Chemicon), donkey anti-goat 546 (Molecular Probe). Nuclei were stained with the nuclear marker TO-PRO3 (Invitrogen).

Laser Capture Microdissection

Frozen sections of rat brains (13 μm thick) at each stage of development (E20, P0, P15, and 6–8 weeks adult) were cut

on Cryostat CM1950 (Leica Microsystems) and mounted on PEN-membrane coated glass slides (Leica Microsystems). After fixation in 70% ethanol and staining with hematoxylin, 1000 cells from meninges and 6–8 weeks adult SVZ were dissected with LMD6000 instrument (Leica Microsystems). Cells were collected in the cap of 0.5 ml tube containing the lysis buffer from Picopure RNA Isolation kit (Arcturus) and RNA extraction was performed according to manufacturer's protocol. First strand cDNA was synthesized with random primers using SuperScript II Reverse Transcriptase (Invitrogen) and used for subsequent qRT-PCR analysis.

Quantitative RT (Reverse Transcription)–PCR Analysis (qRT-PCR)

Total RNA was purified with Trizol reagent (Invitrogen) and retrotranscribed to cDNA by reverse transcriptase AMV contained in the First Strand cDNA Synthesis Kit (Roche). qRT-PCR reactions were carried out in 20 μl total volume containing 10 ng of cDNA (RNA equivalent), 1 μl Power SYBR Green I Master Mix or Taqman Universal PCR Master Mix (Applied Biosystems), 0.4 μM primers forward and reverse or 1/20 Taqman probe. After a starting denaturation for 10 min at 95°C, 40 PCR cycles (15 s 95°C and 1 min 60°C) were carried out on ABI PRISM 7900HT SDS instrument (Applied Biosystems).

Forward and reverse 5_—3_ primer sequences and PCR product lengths were as follows:

Nes: TTGCTTGTGGCCCTGAAAAG, CCAGCTGTGGCA GATGGATT, 129 bp

Sox2: CGCCGAGTGGAAACTTTTGT, CGCGGCCGGTAT TTATAATC, 111 bp

Dcx: AAAGCTTCCCCAACACCTCA, CCATTTGCGTCT TGGTCGTTA, 101 bp

Fgfr1: AAATTCAAATGCCCGTCG, GGCGTAACGAACCT TGTAGCC, 91 bp

Egfr: CCCCACCACGTACCAGATG, GACACACGAGCCG TGATCTGT, 112 bp

Cxcl12 (Sdf1): atcagtgacggtaagccagtca, tgcttttcagccttgcaaca, 145 bp

Cxcr4: cgagcattgccatggaaatat, attgcccactatgccagtcaa, 170 bp

Actb: GGCCAACCGTGAAAAGATGA, GCCTGGATG GCTACGTACATG, 75 bp.

Probe hydrolysis assay for Vim was Rn00579738_m1 (Taqman, Applied Biosystems). The probe signal was normalized to an internal reference and a cycle threshold (Ct) was taken significantly above the background fluorescence. The Ct value used for subsequent calculation was the average of three replicates. The relative expression level was calculated using transcript level of Actb as endogenous reference. Data analysis was done according to the comparative method following the User Bulletin No. 2 (Applied Biosystems).

Western Blot Analysis

Samples were isolated from rat meninges at different developmental stages (E20, P0, P15, and 6–8 weeks adult). Tissue was homogenized in PBS extraction solution with protease inhibitors and extracts were clarified by centrifugation. Protein concentration was determined by using the Bradford protein assay (Sigma). Protein content equivalent to 7 and 10 μg was diluted in loading buffer (Tris-HCl pH 6.8 12 mM, glycerol 20%, SDS 6%, β-mercaptoethanol 28.8 mM, EDTA 4 mM, bromophenol blue 0.2%) and loaded onto constant gradient polyacrylamide gel (10%). Proteins were separated by SDS-PAGE using Biorad electrophoresis system in running buffer (Tris 25 mM, glycine 19.2 mM, SDS 10%), with constant voltage set at 80 V for the entire electrophoresis run. Proteins were transferred onto PVDF membrane, previously equilibrated in methanol, at 60 V in transfer buffer (Tris 25 mM, glycine 19.2 mM, methanol 20%) under refrigerated conditions for 2 h using the Biorad electrophoresis system. Membranes were blocked with 5% BSA and 0.1% Tween-20 in Tris-buffered saline (TBS, pH 7.4) for 1 h and incubated overnight at 4°C with antibodies to DCX and β-actin, diluted in antibody solution (2.5% BSA and 0.1% Tween-20 in TBS pH 7.4): polyclonal rabbit-anti DCX (Cell Signaling; 1:750) (Dellarole and Grilli, 2008) and monoclonal mouse-anti β-actin (Sigma; 1:3000). After washing, membranes were incubated with appropriate HRP secondary antibody diluted in antibody solution for 1 h at room temperature; secondary antibody dilutions were: anti-rabbit IgG HRP conjugated (Chemicon) 1:5000 and anti-mouse IgG HRP (Millipore) 1:2000. Membranes were developed with a chemoluminescence system (ECL Plus, GE Healtcare) and proteins visualized on Hyperfilms (GE Healtcare). Autoradiographs were scanned by Kyocera scanner system.

Transmission Electron Microscopy

For ultrastructure examination, brains from perfused rats were further fixed with 1% glutaraldeheyde in 0.1 M cacodylate buffer pH 7.2 for 30 min, sliced with razor blades, postfixed with 1% OsO4, dehydrated and embedded in Epon (Epon, Electron Microscopy Sciences, USA). Ultrathin sections were with a Philps CM10 transmission electron microscope.

Statistical Analysis

Data were analyzed using GraphPad Prism5 software. Differences between experimental conditions were analyzed using One-Way ANOVA with Tukey *post-hoc* test correction. $P < 0.05$ was considered statistically significant.

Results

Leptomeningeal Cells and Their Proliferation during Development

To analyze the number and the proliferation of cells in the dorsal brain leptomeninges, we studied coronal sections obtained from embryonic (E14, E20), postnatal day 0 (P0) and 15 (P15) and adult (6–8 weeks) rats. The skull and the dura mater were removed from E20 onwards, whereas at E14 the coronal sections included the undifferentiated mesenchymal network of cells from which both the skull and the meninges will be formed. We used laminin, a component of the basal membrane, to visualize the pia mater and spatially distinguish between parenchymal and meningeal nuclei (**Figure 1A**). Immunofluorescence quantitative confocal analysis showed a Gaussian distribution of the number of cells in leptomeninges during the developmental stages,

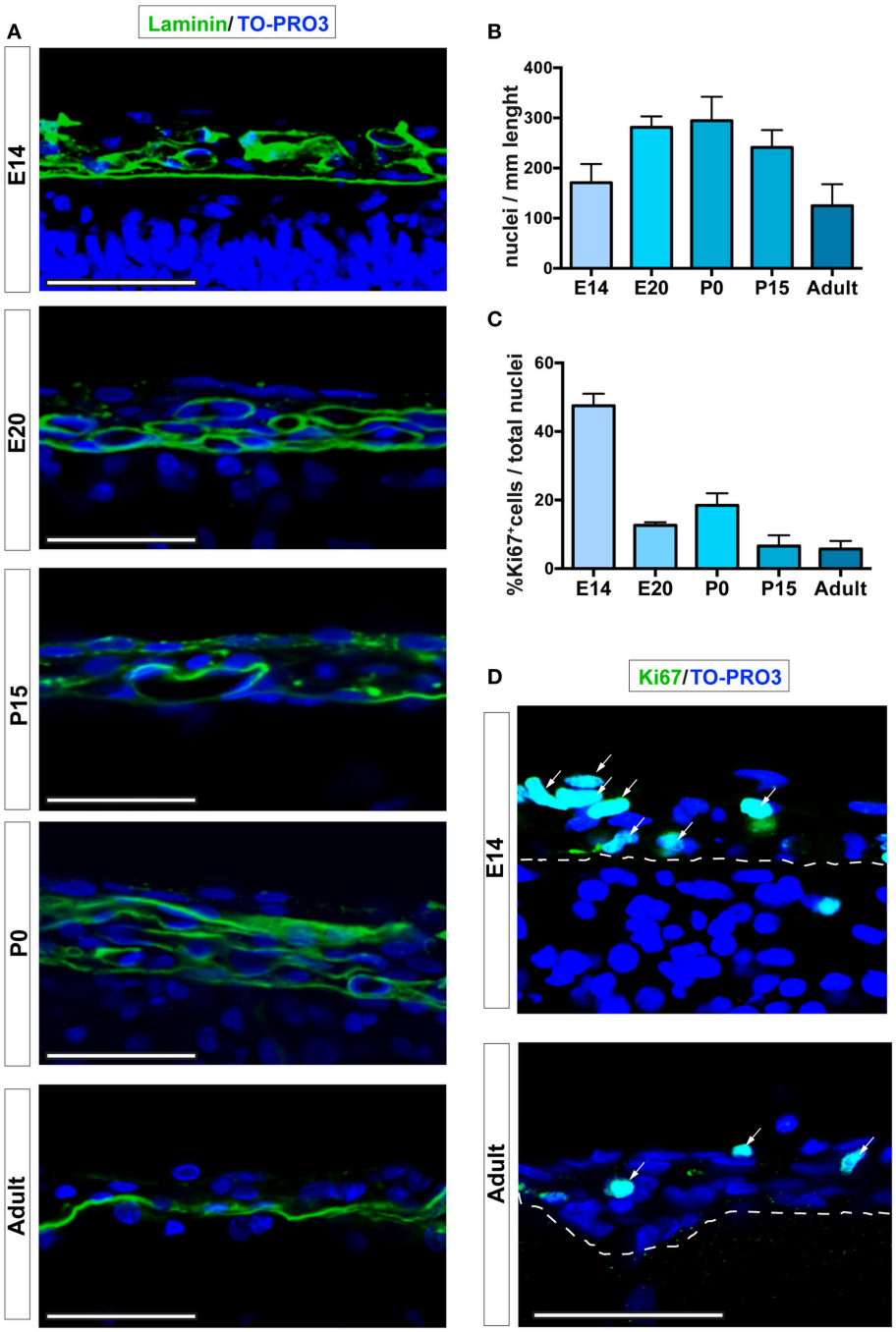

FIGURE 1 | Leptomeningeal cells at different developmental stages. (A) Confocal microscopy analysis of leptomeninges at different stages of development; from top to bottom: E14, E20, P0, P15, adult. Basal lamina of the pia mater was visualized by laminin immunoreactivity (green). **(B)** Quantification of number of meningeal cell nuclei present along 1 mm of brain sections; number of nuclei peaked at P0. **(C)** Percentage of meningeal cells positive for the proliferation marker Ki67. The number of Ki67$^+$ cells is maximum at E14 and decreases going to adulthood. The number of rats analyzed in **(B,C)** was $n = 3$ at E14, $n = 6$ at E20, $n = 3$ at P0, $n = 5$ at P15, and $n = 4$ at adulthood; values represent mean ± SD **(D)**. Confocal microscopy representative images of Ki67$^+$ cells (green) of E14 and 6–8 weeks adult rat brain leptomeninges. Arrows indicate Ki67$^+$ cells, the white dashed line highlights the border between neural parenchyma and meninges. Nuclei are stained with TO-PRO3 (blue). Scale bar: 50 μm.

reaching a peak at P0 (170.8 ± 37.2; 281.3 ± 21.9; 294.6 ± 47.7; 241.1 ± 34.7; 125.0 ± 42.8 nuclei/mm at E14, E20, P0, P15, and 6–8 weeks adult respectively; **Figures 1A,B**).

To further characterize the meningeal tissue, we determined differences in cell proliferation, as defined by expression of the proliferation marker Ki67 (Bullwinkel et al., 2006). The highest

fraction of proliferating leptomeningeal cells was observed at E14 (45.7% ± 2.5 of total nuclei, $n = 3$; **Figures 1C,D**). Although the value decreased with time, the percentage of proliferating cells remained relatively high at all developmental stages as well as in postnatal brains up to 8 weeks (Ki67-positive nuclei: 15.9% ± 2.2; 17.3% ± 7.3; 9.5% ± 9.4; 7.3% ± 6.2 of total nuclei at E20, P0, P15, and 6–8 weeks adult respectively; **Figures 1C,D**). Since distinction of the leptomeninges from dura mater and brain parenchyma is difficult and uncertain before E20, further assessments of the stem cell niche features of the leptomeninges were done starting from this embryonic day.

Leptomeningeal Cells Express Neural Progenitor Markers

The expression of the neural progenitor marker nestin (Decimo et al., 2012b) was analyzed by immunofluorescence confocal microscopy. Nestin is an intermediate filament expressed in all neural precursors and absent in differentiated neural cells (Lendahl et al., 1990). Although the absolute number of nestin-expressing cells in the leptomeninges (identified by their localization above the laminin-reactive pia mater) decreased constantly with age, their proportion remained constant (range from 19.3% ± 5.8 to 23.2% ± 6.5 of total meningeal cells) throughout the analyzed stages (**Figures 2A,D**, **Table 1**). As shown in **Figures 2B,C**, the distribution of nestin-expressing cells appeared as an intricate net of cells adjacent to the basal lamina. The fraction of nestin-positive cells that was also positive for the proliferation marker Ki67 was 15.0% ± 7.4 at E20, peaked at P0 (22.9% ± 10.8) and remained constant later on (11.2% ± 5.4 at P15 and 10.8% ± 4.3 at 6–8 weeks) (**Figures 2A,E**).

We further assessed the presence and distribution of additional neural progenitor markers in the leptomeninges, including vimentin (Stagaard and Mollgard, 1989), Sox2 (Zappone et al., 2000), doublecortin (DCX) (Dellarole and Grilli, 2008), and βIII Tubulin (Tuj1) (Caccamo et al., 1989); for these markers, analysis was extended to 24 weeks-old rats. Vimentin, a type III intermediate filament protein expressed in neural stem cells as well as in mesenchymal cells (Stagaard and Mollgard, 1989; Decimo et al., 2012b), was present in leptomeningeal cells at all stages (**Figure 3A**, **Table 1**). At E20, we observed vimentin- and nestin-double positive cells, while starting from P0, a distinct layer of nestin-positive/vimentin-negative cells appeared. From P15 to adult, nestin-positive and vimentin-positive cells formed distinct layers, however, a fraction of vimentin- /nestin-double positive cells persisted (**Table 1**).

The transcription factor Sox2 is expressed in the neural tube throughout development as well as in postnatal neural progenitors (Zappone et al., 2000). Interestingly, we detected Sox2 immunoreactivity in all the analyzed time points, with higher percentages in embryonic and early postnatal days (**Figure 3B**, **Table 1**). In the adult, Sox2-expressing cells in the meninges were extremely rare, whereas they were located in the brain parenchyma underneath the pia mater basal lamina (**Figure 3B**, **Table 1**).

We also assessed the distribution of neural progenitor markers that have been shown to be expressed at later stages of neuronal precursor differentiation, such as Tuj1 and DCX (Caccamo et al., 1989; Dellarole and Grilli, 2008). No Tuj1-expressing cells were observed in meninges (**Figure 3C**); on the contrary, a limited number of leptomeningeal cells expressed DCX during development up to adult stages (**Figure 3D**, **Table 1**). The presence of DCX protein in meninges at all the developmental stages was confirmed by western blot (WB) analysis: as expected, the amount of DCX present in meninges decreased with age but was still detectable in adult brains (**Figures 3E,F**).

The presence of these neural precursor markers in meninges was further analyzed at the gene expression level. To clearly distinguish leptomeningeal from parenchymal gene expression, we performed laser capture microdissection (LCM) of meningeal tissue and carried out qRT-PCR on the collected samples for gene expression analysis (**Figures 4A,B**); SVZ tissue isolated from 6 to 8 weeks adult rats was used as positive control. Consistently with the immunofluorescence and WB analysis, we detected expression of nestin, vimentin, Sox2 and DCX genes at all stages including adulthood (**Figure 4C**). We observed that leptomeningeal gene expression levels of nestin and vimentin genes were comparable to SVZ, while Sox2 and DCX genes were expressed at lower levels, suggesting differences in cellular composition between the two tissues.

These results suggest that leptomeninges host precursor cells expressing nestin, vimentin, Sox2 and DCX during development. Nestin expressing meningeal cells appeared to be abundant and to retain proliferation properties from embryo until adulthood.

Major Extracellular Components of the Meningeal Tissue during Development

Neural stem cell niches are characterized by the presence of extracellular matrix components and chemotactic factors (Kerever et al., 2007; Kokovay et al., 2010). Accordingly, we assessed the presence of laminin and N-sulfated heparan sulfate (N-sulfated HS), a member of the glycosaminoglycan family that has been shown to bind and concentrate growth factors, including FGF2 and epidermal growth factor (EGF) (Yayon et al., 1991; Mercier and Arikawa-Hirasawa, 2012). Immunoreactivities for laminin and N-sulfated HS were observed by confocal microscopy in brain leptomeninges at all the developmental stages analyzed (**Figure 5A**). Interestingly, both laminin and N-sulfated HS were present in vascular basement membranes and in fractones (Mercier et al., 2002), specialized extracellular matrix structures appearing as series of immunoreactive puncta aligned along the meninges (arrows in **Figure 5A**). Fractones were also observed at the ultrastructural level (**Figure 5B**), where they appeared as electrondense material formed by extravascular basal lamina with typical folds and tube-like morphology and measuring 5–10 μm in length and 1–4 μm in diameter (**Figure 5B**). Meningeal fractones were similar to fractones described in the SVZ (Mercier et al., 2002), suggesting that meninges are endowed, during development and in adulthood as well, with extracellular matrix organized in specific structures that promote heparin-binding growth factor activity and cell proliferation. Indeed, growth factors relevant for neural development, such as FGF2 and heparin binding-EGF,

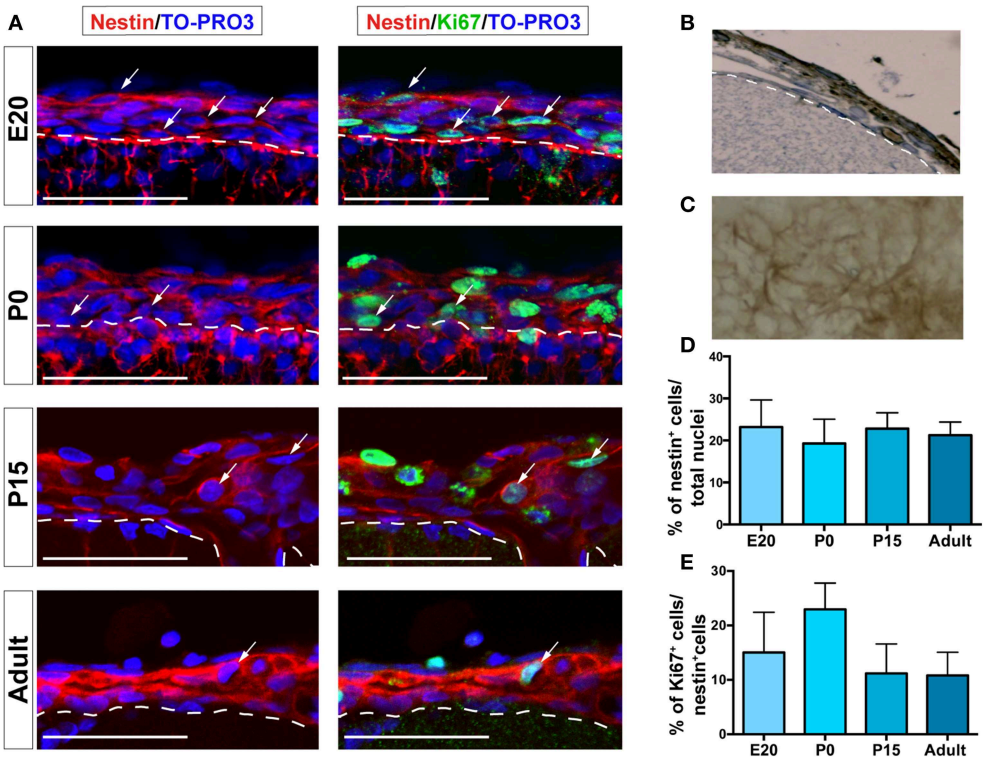

FIGURE 2 | Nestin$^+$ and Ki67$^+$ cells are present in leptomeninges. (A) Immunostaining of nestin (red, left column) and nestin (red)/Ki67 (green, right column) at different stages of development; from top to bottom: E20, P0, P15, adult. Confocal microscopy analysis revealed that nestin$^+$ cells (red) are present in the leptomeninges from embryonic stage E20 up to adulthood. Nuclei are stained with TO-PRO3 (blue). Scale bar: 50 μm. **(B,C)** Immunoperoxidase staining (brown) with anti-nestin antibody of brain sections. **(B)** Coronal section; the white dashed line highlights the border between neural parenchyma and meninges. **(C)** *En face* view showing nestin$^+$ cells as an intricate net covering the brain. **(D)** Quantification of nestin$^+$ cells normalized for the total number of nuclei in meninges. **(E)** Percentage of nestin$^+$/Ki67$^+$ cells; the values are normalized for the number of nestin$^+$ cells. In **(D,E)**, values are mean ± SD.

have been found in meninges (Nakagawa et al., 1998; Mercier and Hatton, 2001).

In line with these findings, we detected gene expression of the growth factor receptors FGFR1 and EGFR in leptomeninges at all-time points of analysis (**Figure 5C**). Moreover, the chemotactic factor SDF-1 and its receptor CXC chemokine receptor 4 (CXCR4) were also expressed in leptomeninges at all the developmental stages analyzed (**Figure 5C**). SDF-1 and its receptor CXCR4 are known to be involved in homing, movement, proliferation and differentiation of progenitor cells (Kokovay et al., 2010), further indicating that leptomeninges may be a niche for neural progenitors.

Collectively, these data suggest that the extracellular components of the meninges form a microenvironment favoring homing and proliferation of precursor cells.

Discussion

Previous works described the presence in adult meninges of a stem cell-like population that reacts to CNS injury by displaying the hallmarks of a neural stem cell niche: activation, increased proliferation and migration to the lesioned parenchyma (Decimo et al., 2011; Nakagomi et al., 2011, 2012; Ninomiya et al., 2013; Kumar et al., 2014). Moreover, a population of nestin-positive cells could be extracted from meningeal tissue, cultured *in vitro* and showed neural differentiation potential *in vitro* and after transplantation *in vivo* (Bifari et al., 2009; Nakagomi et al., 2011). These observations led us to further investigate whether meninges possess the features described for canonical neural stem cell niches (Bjornsson et al., 2015) and whether these features also persist at the end of the developmental period.

Cell Expressing Neural Precursor Markers Are Retained in Meninges

The neural stem cell niche is a tissue microenvironment capable of hosting and maintaining neural progenitor cells for the lifetime (Scadden, 2006; Decimo et al., 2012b). It ensures a unique microenvironment where interactions between cells, extracellular matrix molecules (ECM) and soluble signals, provide the proper control of neural precursor renewal and differentiation (Scadden, 2006; Decimo et al., 2012a,b; Bjornsson et al., 2015).

All these features are expressed and maintained in adulthood in the most studied neurogenic niches, i.e., the subventricular zone (SVZ). At this site, different cell types are present, including quiescent NSCs, transient amplifying precursors and committed neuroblasts, each expressing specific sets of markers (Doetsch

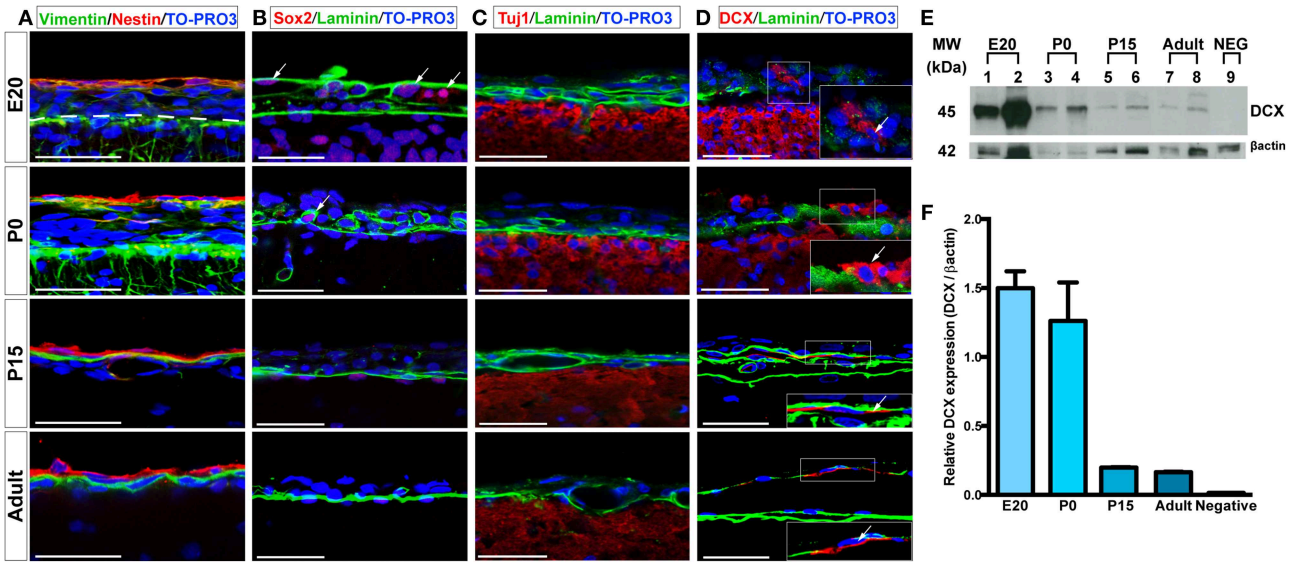

FIGURE 3 | Expression of neural progenitors markers in the leptomeninges. (A–D) Confocal microscopy images of leptomeninges in brain coronal sections stained with vimentin (green)/nestin (red) (A), Sox2 (red)/laminin (green) **(B)**, Tuj1 (red)/laminin (green) **(C)**, and DCX (red)/laminin (green) **(D)** at different stages of development; from top to bottom: E20, P0, P15, adult. Arrows in **(B,D)** point to $Sox2^+$ and DCX^+ cells respectively. Nuclei are stained with TO-PRO3 (blue). Scale bar: 50 μm. **(E)** Western Blot of meninges lysates. 7 μg of total protein lysate were loaded in lanes 1, 3, 5, 7, 9 and 10 μg in lanes 2, 4, 6, 8. Lanes 1–2: lysates from E20 meninges. Lanes 3–4: lysates from P0 meninges. Lanes 5–6: lysates from P15 meninges. Lanes 7–8: lysates from adult meninges. Lane 9: lysates from P0 meninges as negative control for the secondary antibody. Numbers on the left indicate molecular masses in kilodaltons (kDa). **(F)** Densitometric analysis of relative protein levels shown in **(E)**. DCX expression was normalized for β-actin expression. DCX relative expression is high in E20 and P0 meningeal lysates and persists in P15 and adult meningeal lysates.

TABLE 1 | Quantitative analysis of neural precursor markers in leptomeninges at different developmental stages.

	$Vimentin^+$ cells	$Nestin^+$ cells	$Vimentin^+/Nestin^+$ cells	DCX^+ cells	$Sox2^+$ cells
E20	25.7% ± 4.8 ($n = 4$)	23.2% ± 6.5 ($n = 4$)	12.9% ± 5.1 ($n = 3$)	10.5% ± 4.4 ($n = 4$)	2.6% ± 1.1 ($n = 3$)
P0	20.6% ± 3.9 ($n = 4$)	19.3% ± 5.8 ($n = 4$)	8.2% ± 1.3 ($n = 4$)	13.0% ± 4.1 ($n = 4$)	4.2% ± 3.4 ($n = 3$)
P15	24.8% ± 4.5 ($n = 5$)	22.8% ± 3.8 ($n = 3$)	7.8% ± 1.9 ($n = 3$)	4.8% ± 5.2 ($n = 4$)	0.2% ± 0.3 ($n = 3$)
Young adult (6–8 weeks)	31.0% ± 0.8 ($n = 3$)	21.3% ± 3.1 ($n = 3$)	8.2% ± 1.8 ($n = 3$)	1.4% ± 1.1 ($n = 3$)	0.5% ± 0.5 ($n = 3$)
Mature adult (24 weeks)	32.2% ± 5.4 ($n = 3$)	17.7% ± 1.4 ($n = 3$)	5.5% ± 3.1 ($n = 3$)	1.4% ± 0.7 ($n = 3$)	0.3% ± 0.6 ($n = 3$)

Quantifications were performed by counting cells lying above the pial basal lamina (visualized by laminin immunoreactivity). Numbers are expressed as the percentage of positive cells on the total number of cells counted. Data are mean ± SD; n = number of animals analyzed.

et al., 1997). With this study we show that leptomeninges harbor a population of cells expressing the undifferentiated neural precursor markers nestin, vimentin and Sox2. Approximately 20% of the leptomeningeal cells expressed nestin and roughly 15% of those cells were in the active phase of the cell cycle in all the stages analyzed. At all time-points, a small fraction of meningeal cells also expressed DCX, a microtubule-associated protein expressed by neuronal precursor cells and immature neurons in embryonic and adult cortical structures. Thus, similar to the SVZ, leptomeninges host a subset of cells expressing markers of undifferentiated, proliferating and differentiating neural precursors and this set of cells persists in adulthood. Thus, meninges may represent a functional niche for progenitors during embryonic development and in adulthood.

Although leptomeninges share several features of the SVZ niche, our data also highlight quantitative differences in Sox2 and DCX gene expression levels between these two tissues, possibly reflecting differences in cell composition and in functional significance for brain homeostasis.

Leptomeninges Possess Molecules Necessary to Form a Microenvironment Favoring Proliferation and Homing of Precursor Cells

In SVZ distinct ECM components and chemotactic factors have been described, including FGF2 and epidermal growth factor (EGF) (Yayon et al., 1991; Mercier and Arikawa-Hirasawa, 2012), as well as components of chemoattractant signaling systems such as SDF-1 and its receptor CXCR4. Members of this signaling machinery act in concert, as shown by SDF-1-induced stimulation in EGFR-expressing cells of movement toward the blood vessel surface, proliferation and generation of transient amplifying cells (Kokovay et al., 2010).

Our gene expression data confirm that similar signaling machinery is present in meninges. Indeed, we found

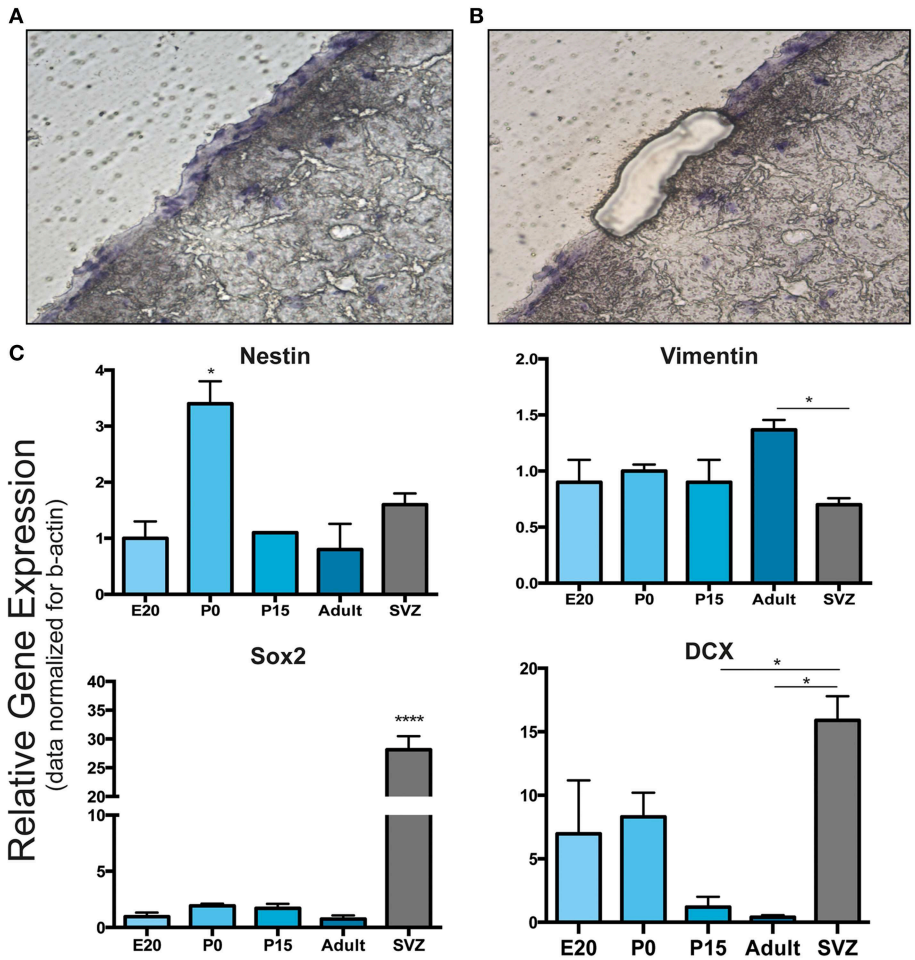

FIGURE 4 | Laser capture microdissection and gene expression analysis of leptomeningeal cells. (A,B) Laser capture microdissection (LCM) was performed to distinguish leptomeningeal from parenchymal gene expression. **(A)** Shows a coronal brain section with the entire meningeal layer before LCM. **(B)** Shows the same section after meningeal dissection. From each stage of development (E20, P0, P15, adult), at least 1000 cells were collected from meningeal tissue **(B)**. **(C)** qRT-PCR on collected samples was performed for gene expression analysis. As expected from immunofluorescence and WB analysis, we detected expression of nestin, vimentin, Sox2 and DCX genes. Expression of all these neural precursor-related genes persisted up to adulthood. SVZ samples from 6 to 8 weeks adult rats were used as positive control. $^{*}p < 0.05$; $^{****}p < 0.0001$. Values are mean ± SEM of 3 replicates.

expression of the growth factor receptors FGFR1 and EGFR in leptomeninges, as well as of the homing chemotactic factor SDF-1 and its receptor CXCR4 from embryonic to adult stages. Our data are in line with published results showing that meninges are highly responsive to several mitogens, including EGF, FGF-2 and BDNF (Day-Lollini et al., 1997; Parr and Tator, 2007). Moreover, SDF-1 secreted by meningeal cells acts as chemotactic factor on neural cells (Borrell and Marin, 2006). Interestingly, modulation of this chemoattractant signaling system was observed following spinal cord injury (increase of CXCR4/SDF-1 ratio) (Decimo et al., 2011).

The persistent expression in meninges of these important signals for proliferation, homing and migration of neural progenitors suggests that cellular dynamics in the CNS are complex and that, depending on the needs of the brain parenchyma, the meningeal niche may adapt its signals promoting either proliferation, migration or homing. In this context, it is important to note that our data indicate the presence of fractones at all stages of life, including both development and adulthood. Fractones are specialized extracellular matrix structures that appear to bind and concentrate important regulators of proliferation and migration (Kerever et al., 2007; Mercier and Arikawa-Hirasawa, 2012). These N-sulfated HS structures have been described in detail both in rodent and human brains: they are present associated to well-described sites of adult neurogenesis such as the SVZ and the hippocampus and appear to form a continuum across these neurogenic niches connecting them to the olfactory bulb, the rostral migratory stream, the sub-callosum, the subcapsule zones and the meninges (Mercier and Arikawa-Hirasawa, 2012). This confirms that meninges have the potential to connect different portions of the brain.

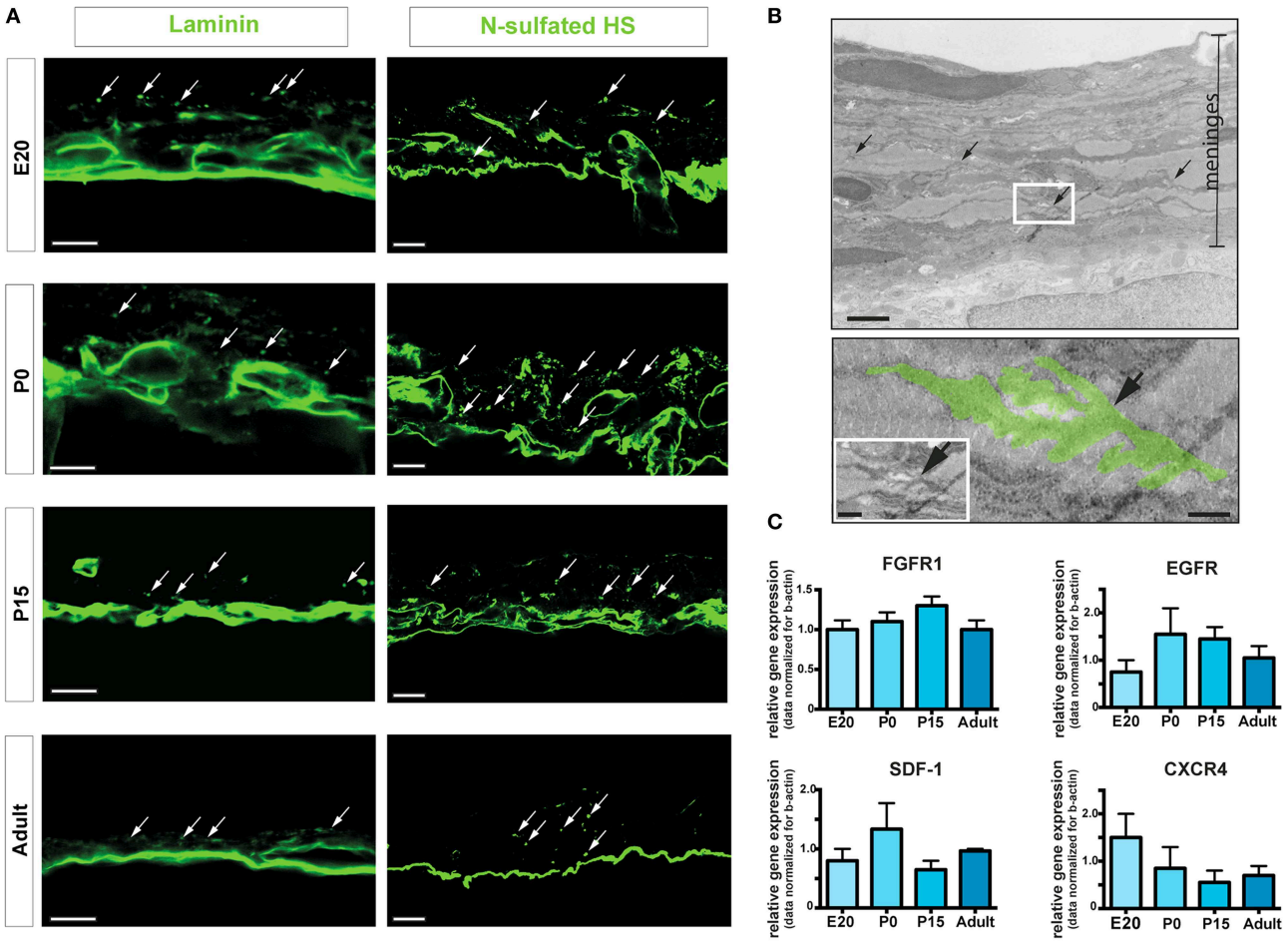

FIGURE 5 | ECM components and fractones. (A) Confocal images of meninges in brain coronal sections showing the presence of immunoreactivities for laminin and N-sulfated HS. Dot-like aggregates (arrows) suggest organization of fractones in the leptomeninges. Scale bar: 10 μm. **(B)** Transmission electron microscopy representative image of P15 rat meninges. The white rectangle in the upper picture is enlarged in the lower frame; colored area highlights a fractone. Scale bar: 5 μm upper panel; 0.5 μm bottom panel and 1 μm in the insert. **(C)** Relative gene expression of FGFR1, EGFR, SDF1, and CXCR4 of rat leptomeninges at E20, P0, P15, and 6–8 weeks adult. Values are mean ± SEM of 3 replicates.

In line with the idea that meninges play a pivotal role in guiding stem cells migration in the brain, are our observations that transplanted leptomeningeal stem cells accumulate in meninges following injection in the third ventricle of adult animals [unpublished observations] and the finding of ectopic colonies at the pial surface of the spinal cord following embryonic neural stem cells transplantation at the site of injury (Steward et al., 2014).

Conclusion

This study provides a new and accurate description of the molecular and cellular aspects of meninges related to their newly identified function of niche for neural progenitor/stem cells. We add to previous information the notion that this niche is indeed present and potentially active at all stages of development and in adult life as well. The identification of receptors for trophic factors, of ECM components and chemotactic factors known to be involved in homing, movement, proliferation and differentiation of progenitor cells strengthens the idea that the niche function of meninges is not limited to conditions associated to diseases, such as injury or ischemia (Decimo et al., 2011; Nakagomi et al., 2012).

Our description of the molecular and cellular properties of the meningeal niche in healthy animals calls for a physiological function of this progenitor niche. The notion that neurogenesis may occur in response to physiological and not just pathological stimuli is well accepted (Kempermann et al., 1997); although the earliest and the most abundant information have been obtained from well identified structures including SVZ and hippocampus, data indicate that neurogenesis may also occur in response to physiological stimuli at sites that are distant from those classical niches (Dayer et al., 2005). In this context, we propose that meninges may be a wide-spread niche from where neurogenesis may be induced on demands following physiological stimuli; alternatively, or in addition, meninges may serve as a highway

for delivery to distant sites of neural precursors newly generated in classical neurogenic niches. Further studies tracing the fate of meningeal cells are therefore needed to clarify the functional significance of this newly discovered niche and to determine the potential role of meninges in brain homeostasis.

Author Contribution

All authors performed research and/or analyzed data; FB, VB, GF, and ID designed research and wrote the paper. All authors discussed the results and commented on the manuscript.

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Osteogenesis and neurogenesis: a robust link also for language evolution

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This paper seeks to contribute to the characterization of the relation between osteogenesis and neurogenesis by approaching it from the field of the neurobiology of language and cognition; specifically, from an evolutionary perspective. It is difficult to ascertain how the hominin brain changed to support modern language and cognitive abilities because we can only rely on skull remains. But insights can be gained from fossils because the brain and the skull exhibit a tight relationship. Skull shape and brain shape and connectivity influence one another (Roberts et al., 2010; Lieberman, 2011). Craniofacial anomalies and cognitive disorders frequently co-occur (see Boeckx and Benítez-Burraco, 2014a for review). So, "osteo" considerations can shed light on "neuro" considerations (and vice versa). Importantly, main differences between anatomically-modern humans (AMHs) and Neanderthals pertain not to the brain size, but to the more globularized headshape of the former (Bruner, 2004). Globularity results from an AMH-specific developmental trajectory after birth, at a stage when the brain is the primary determinant of skull shape (Gunz et al., 2010). Globularization is not just a morphological change of the skull. On the contrary, factors giving rise to globularity also have important neurofunctional consequences. The hypothesis we have explored in our recent work is that the rewiring of the hominin brain associated to globularization brought about our most distinctive mode of cognition (see Boeckx and Benítez-Burraco, 2014a for details).

In a series of related papers (Boeckx and Benítez-Burraco, 2014a,b; Benítez-Burraco and Boeckx, 2015) we have examined closely some of the most critical genes that may contribute to skull globularity and that have been selected in AMHs. These also contribute significantly to neurogenesis, as well as to neural specification, arealization of the neo-cortex, neuronal interconnection, and synaptic plasticity. Eventually, the very osteogenic signals that help build our distinctive skull also contributes to build our distinctive mode of brain organization underlying our mode of cognition and language abilities.

Our main candidate is *RUNX2*. A selective sweep in this gene occurred after our split from Neanderthals (Green et al., 2010). It is a candidate for cleidocranial dysplasia (Yoshida et al., 2003) and controls the closure of cranial sutures (Stein et al., 2004). Together with *DLX5* and *TLE1* it regulates the integration of the parietal bone (Depew et al., 1999; Stephens, 2006), a "hotspot" for globularization (Bruner, 2004). However, it is also involved in the development of the hippocampal GABAergic neurons as part of the GAD67 regulatory network (Pleasure et al., 2000; Benes et al., 2007). Moreover, it seems to be also involved in the development of thalamus (Reale et al., 2013). Its mutations cause mental diseases in which our mode of cognition is impaired (Talkowski et al., 2012; Ruzicka et al., 2015). Importantly, *RUNX2* is deeply implicated in the regulation of osteocalcin (Paredes et al., 2004) and osteopontin (Shen and Christakos, 2005), which are important for both bone formation and brain organization (e.g., osteopontin-deficient mice suffer from thalamic neurodegeneration; Schroeter et al., 2006).

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Interestingly, *RUNX2* is functionally connected to many genes that are important for brain and language development, but also to bone formation. To begin with, *RUNX2* is a regulatory target of AUTS2 (Oksenberg et al., 2014). *AUTS2* is among the genes found to be differentially expressed after *RUNX2* transfection in neuroblastomic cell lines (Kuhlwilm et al., 2013). The first half of *AUTS2* displays the strongest signal of positive selection in AMHs compared to Neanderthals (Green et al., 2010). Mutations in *AUTS2* give rise to a host of cognitive impairments (see Oksenberg and Ahituv, 2013 for review). Interestingly, these routinely co-occur with skeletal abnormalities and/or dysmorphic features (Beunders et al., 2013). AUTS2 interacts with some other proteins like TBR1, RELN, SATB2, GTF2I, ZMAT3, or PRC1 that play a key role at the brain level and have been related to ASD and other developmental disorders affecting cognition and language (Oksenberg and Ahituv, 2013). Some of them directly interact with RUNX2.

For example, *RUNX2* directly interacts with *SATB2* (Hassan et al., 2010), a gene that regulates stereotypic projections in the cortex (Srinivasan et al., 2012). This gene has been related to ASD, intellectual disability, and language delays, as well as craniofacial defects (Liedén et al., 2014) and plays a key role in osteoblast differentiation, palate formation, and craniofacial development (Zhao et al., 2014). Crucially, the interaction between SATB2 and *RUNX2* is very relevant during osteogenesis (Hassan et al., 2010; Gong et al., 2014). Specifically, several micro-RNAs (including miR-205 and miR-31), SATB2, RUNX2, osteopontin and osteocalcin interact complexly to modulate the differentiation of bone mesenchymal stem cells into osteoblasts (Deng et al., 2013; Hu et al., 2015). Interestingly, in the neural *satb2* expression depends on both Bmp and Shh (Sheehan-Rooney et al., 2013), which are genes we have highlighted in our previous work. Moreover, SATB2 represses the expression of *HOXA2* (Ye et al., 2011), which is one of the targets of the famous "language gene" *FOXP2* (Konopka et al., 2009). *HOX2A* is involved in both the brain and bone formation. Accordingly, it contributes to the hindbrain patterning (Miguez et al., 2012), acting upstream the guidance signals Robo1, Robo2, Slit1, and Slit2 in the anteroposterior migration of pontine neurons (Geisen et al., 2008). However, it also encodes an inhibitor of bone formation (Dobreva et al., 2006; Ye et al., 2011), which controls the morphology of the skeleton (Tavella and Bobola, 2010). Interestingly also, the activation of *Hoxa2* in the neural crest downregulates Bmp antagonists and leads to severe craniofacial and brain defects (Garcez et al., 2014).

Additionally, RUNX2 interacts (via FOXO1) with *DYRK1A* (Huang and Tindall, 2007), a gene located within the Down Syndrome Critical Region on chromosome 21. This gene has been linked to microcephaly, facial dysmorphism, mental retardation, and absence of speech (van Bon et al., 2011; Courcet et al., 2012). *DYRK1A* has been shown to be involved in bone homeostasis as an inhibitor of osteoclastogenesis (Lee et al., 2009). DYRK1A is also of interest because it phosphorylates SIRT1, which controls neural precursor activity and differentiation (Saharan et al., 2013). SIRT1 both upregulates *RUNX2* and deacetylates RUNX2, ultimately promoting osteoblast differentiation (Shakibaei et al., 2012; Srivastava et al., 2012), an effect which is also due to its effects on β-catenin and FoxO in osteoblast progenitors (Iyer et al., 2014). Importantly, resveratrol-induced SIRT1 activation promotes neuronal differentiation of human bone marrow mesenchymal stem cells (Joe et al., 2015). Finally, *RUNX2* is also functionally related (via AUTS2) to *CBL*, in turn linked to Noonan syndrome-like disorder, a condition involving facial dysmorphism, a reduced growth, and several cognitive deficits (Martinelli et al., 2010). This gene, which encodes an inhibitor of osteoblast differentiation and promotes the degradation of Osterix (Choi et al., 2015), is located within a region showing signals of a strong selective sweep in AMHs compared to Altai Neanderthals (Prüfer et al., 2014).

RUNX2 is also functionally directly linked to the FOXP2 and ROBO1 interactomes (see Boeckx and Benítez-Burraco, 2014b for details), which are related to language disorders and vocal learning (Graham and Fisher, 2013; Pfenning et al., 2014). To begin with, a direct interaction between *RUNX2* and *FOXP2* has recently been experimentally demonstrated (Zhao et al., 2015b). This finding was further reinforced in Gascoyne et al. (2015), who added *FOXP2* to the list of established osteoblast and chondrocyte transcription factors such *RUNX2*, *SP7*, and *SOX9*. In fact, *FOXP2* seems to regulate both bone formation (it regulates endochondral ossification) (Zhao et al., 2015b), and the fate of neural stem cells during corticogenesis (MuhChyi et al., 2013). As for the ROBO suite, some members like *HES1* and *AKT1* are functionally related to RUNX2. *HES1* is needed for the correct functioning of the Slit/Robo signaling pathway during neurogenesis (Borrell et al., 2012) and plays a role as well in the development of both GABAergic and dopaminergic neurons. *Hes1* silencing promotes bone marrow mesenchymal stem cells to differentiate into GABAergic neuron-like cells *in vitro* (Long et al., 2013). Moreover, Hes1 modulates skeletal formation and pathogenesis of osteoarthritis via calcium/calmodulin interaction (Sugita et al., 2015). In turn *AKT1* is a critical mediator of growth factor-induced neuronal survival (Dudek et al., 1997). In mice mutations in *Akt1* and *Akt2* impair bone formation (Peng et al., 2003). *AKT1* has recently been shown to coordinate the bone-forming osteoblasts and bone-resorbing osteoclasts, a process important for maintaining skeletal integrity (Akt1 deficiency impairs osteoclast differentiation and diminishes the rate of proliferation of osteoblast progenitors) (Mukherjee et al., 2014).

Other bone morphogenetic factors may well play a key role in the emergence of our language-readiness and our globular brain. Among them we wish highlight the DLX suite (particularly, *DLX1, DLX2, DLX5,* and *DLX6*) and the BMP suite (specifically, *BMP2* and *BMP7*): most of them also interact with *RUNX2*. Consider, e.g., *DLX2*. It is involved in craniofacial development (Jeong et al., 2008), but it is also needed for neocortical and thalamic growth (Jones and Rubenstein, 2004). Mutations in this gene affect craniofacial and bone development (Kraus and Lufkin, 2006), but also cognitive development (Liu et al., 2009). It also takes part in the regulation of neuronal proliferation within the cortex (McKinsey et al., 2013). Concerning the BMP proteins, both BMP2 and BMP7 interact with RUNX2 and both of them play a role in bone and brain formation. *BMP2* promotes the differentiation of mesenchymal cells into bone cells (Dwivedi

et al., 2012), but it is also needed for normal neurogenesis in the ganglionic eminences and correct cortical neurogenesis (Shakèd et al., 2008). In mice Bmp2 (and also Bmp7) upregulates *Dlx1*, *Dlx2*, *Dlx5*, and *Runx2* (Bustos-Valenzuela et al., 2011). Much like *BMP2*, *BMP7* is involved in osteogenesis (Cheng et al., 2003) and skull and brain development (Segklia et al., 2012). Mutations in this gene give rise as well to developmental delay and learning disabilities (Wyatt et al., 2010).

We further believe that the genetic aspects highlighted here may contribute not only to gain a better understanding of the way in which both aspects of our modernity emerged and interact, but specifically to tune the crosstalk between the osteogenic and neurogenic stem cell niches. Zhao et al. (2015a) have recently identified *Gli1*+ cells within the suture mesenchyme as the main mesenchymal stem cell population for craniofacial bones. Ablation of these *Gli1*+ cells leads to craniosynostosis, known to be associated with cognitive deficits (Starr et al., 2007), and arrest of skull growth. Not surprisingly, Gli1 is known to regulate *Runx2* (Kim et al., 2013). In turn, Gli1 transcriptional activity is regulated by Dyrk1a (Mao et al., 2002), whereas Hes1 directly modulates *Gli1* expression (Schreck et al., 2010). Moreover, *Gli1* is the direct response gene of Shh (Liu et al., 1998). The Shh-Gli1 pathway has been shown to regulate brain growth (Dahmane et al., 2001; Ruiz i Altaba et al., 2002; Corrales et al., 2004), and to control thalamic progenitor identity and nuclei specification (Vue et al., 2009), as well as the development of the cerebellum (Lee et al., 2010). It may also be the case that *FoxP2* lies downstream of *Shh*, as suggested by Scharff and Haesler (2005), who observed that the zinc finger motif of FoxP2 is highly homologous to those of the major Shh downstream transcriptional effectors, particularly, of Gli1, Gli2, and Gli3. Moreover, balanced Shh signaling is required for proper formation and maintenance of dorsal telencephalic midline structure (Himmelstein et al., 2010). Dysregulation of the neural stem cell pathway Shh-Gli1 has been observed in autoimmune encephalomyelitis and multiple sclerosis (Wang et al., 2008). As a matter of fact, a GLI1-p53 inhibitory loop controls neural stem cell (Stecca and Ruiz i Altaba, 2009). Most interestingly for us, Marcucio et al. (2005) have shown that excessive Shh activity, caused by truncating the primary cilia on cranial neural crest cells, causes hypertelorism, and frontonasal dysplasia. This condition has been shown to be associated to mental retardation, lack of language acquisition, and severe central nervous system deficiencies (Guion-Almeida and Richieri-Costa, 2009). The latter example appears to lend credence to our final claim that language and cognition are intimately related to the molecular mechanisms associated with mesenchymal stem cell and neural stem cell populations.

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