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REVIEW ARTICLE

Sexual dimorphisms in adult human neural, mesoderm-derived, and neural crest-derived stem cells

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Sexual dimorphisms contribute, at least in part, to the severity and occurrence of a broad range of neurodegenerative, cardiovascular, and bone disorders. In addition to hormonal factors, increasing evidence suggests that stem cell-intrinsic mechanisms account for sex-specific differences in human physiology and pathology. Here, we discuss sex-related intrinsic mechanisms in adult stem cell populations, namely mesoderm-derived stem cells, neural stem cells (NSCs), and neural crest-derived stem cells (NCSCs), and their implications for stem cell differentiation and regeneration. We particularly focus on sex-specific differences in stem cell-mediated bone regeneration, in neuronal development, and in NSC-mediated neuroprotection. Moreover, we review our own recently published observations regarding the sex-dependent role of NF-κB-p65 in neuroprotection of human NCSC-derived neurons and sex differences in NCSC-related disorders, so-called neurocristopathies. These observations are in accordance with the increasing evidence pointing toward sex-specific differences in neurocristopathies and degenerative diseases like Parkinson's disease or osteoporosis. All findings discussed here indicate that sex-specific variability in stem cell biology may become a crucial parameter for the design of future treatment strategies.

Keywords: mesoderm-derived stem cells; neural crest-derived stem cells; neural stem cells; neurocristopathies; neuroprotection; NF-κB; sex-specific differences; stem cells

Differences between the sexes are present in the adult organism and can be observed even in the early stage of mammalian embryonic development, as suggested for the first time in 350 BC by Aristotle [1]. Accordingly, *ex vivo* generated male mammalian embryos used for *in vitro* fertilization were reported to show increased growth in comparison with their female counterparts, suggesting the presence of hormonal-independent genetic difference between the sexes even in early development [2–4]. In addition, early female mammalian embryos show higher expression of genes involved in detoxification of reactive oxygen species [5]. Differences between the sexes are also observable in the adult organism, as in terms of the increased longevity of female individuals and sex-specific differences in human diseases. Here, sex differences are increasingly noticed to attribute at least in part to the occurrence and severity of human diseases, particularly regarding neurodegenerative [6,7], cardiovascular [8,9], and endocrine disorders [10] as well as sepsis [11] and bone defects [12]. As the major developmental and regenerative unit of the human

Abbreviation
ASCs, adipose tissue-derived stem cells; EDN3, endothelin 3; EDNRB, endothelin receptor type B; GDNF, glial cell line-derived neurotrophic factor; HD, Hirschsprung disease; ITSCs, inferior turbinate stem cells; MDSCs, muscle-derived stem cells; MSCs, mesenchymal stem cells; NC, neural crest; NCSCs, neural crest-derived stem cells; NSCs, neural stem cells; PNS, peripheral nervous system.

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organism, stem cell behavior, differentiation, and regenerative potential also critically depend on the sex of the individual [13–16]. Mechanistically, differential effects of sex-specific hormones like estrogen and testosterone were reported to account, at least in part, for differences in stem cell behavior between the sexes (reviewed in [16]). For instance, estrogen was shown to modulate the expression of estrogen receptor alpha and beta as well as osteogenic activity of mouse mesenchymal stem cells (MSCs) in a mouse model of osteoporosis [17]. Regarding the ectodermal lineage, Marin-Husstege and coworkers reported proliferation and maturation of rodent oligodendrocyte progenitor to be differentially regulated by male and female sex steroid hormones [18]. Estrogen was further demonstrated to directly increase the self-renewal of hematopoietic stem cells in female human individuals and particularly during pregnancy. Notably, female human HSCs and their progeny divided more frequently than male HSCs even under basal conditions [15,19,20]. Interestingly, estrogen was described to directly activate telomerase *via* ER-mediated transcription of TERT in human breast cancer MCF-7 cells [21]. In accordance with these observations, Dulken and Brunet suggested a mechanism of age-delay in stem cells within a female environment [22].

Next to the presence of sex steroid hormones (see [16] for detailed review), stem cell-intrinsic mechanism is increasingly noticed to account for sex-specific differences in the regeneration capability and behavior of stem cells. In this regard, Tamaki *et al.* [23] observed an increased survival of human hematopoietic stem cell transplantations from maternally donated recipients compared to paternal transplantations. Accordingly, sex-dependent cell-intrinsic mechanisms were shown to play a significant role in the closure of wounded tissue in the mammalian system. Blankenhorn *et al.* [24] reported a faster and more complete regrowth of cartilage, skin, and hair follicles in an ear pinna regeneration model in female mice compared to their male counterparts. Notably, castration of males led to better healing, although ovariectomy did not result in reduced healing efficiency. Interestingly, in sheep and rat models ovariectomy was also reported to closely mimic osteoporosis, a multifactorial disease frequently observed in postmenopausal women [25,26]. Reasonable underlying mechanisms could be either NF-κB-mediated osteoclastic activity, which is induced by increased levels of TNFα after ovariectomy [27] or loss of MSCs [28]. In line with the findings by Blankenhorn *et al.*, reperfusion of infarcted rat hearts with female MSCs resulted in significantly increased recovery of left ventricular developed pressure compared to infarcted hearts treated with male MSCs [13]. Female muscle-derived stem cells (MDSCs) were further shown to regenerate skeletal muscle more efficiently than male MDSCs *in vivo*, based on innate sex-related but hormone-independent differences in responses to stress [14]. Thus, intrinsic mechanisms defined by the sex seem a biologically relevant aspect of the protective and regenerative power of adult stem populations [13,14]. In accordance with these findings in adult stem cells, hormonal-independent differences in stem cell behavior between the sexes also exist in early development. Here, a global gene expression analysis of murine embryonic neural stem cells (NSCs) revealed 103 differentially expressed transcripts between male and female NSCs prior to gonadal-derived hormonal surges. Bramble *et al.* [29,30] reported these transcriptional sex differences to be involved in cellular replication, indicating a differential regulation of proliferative and stemness states between male and female NSCs during development. These observations in the adult organism as well as within early mammalian development evidence the existence of stem cell-intrinsic sex differences in addition to already described effects of hormones.

In the present review, we will highlight and further discuss intrinsic sex-related mechanisms in adult stem cells, particularly focusing on its implications for neuronal and osteogenic differentiation processes and endogenous stem cell-based regeneration. Among the adult stem cell population discussed here, we will put particular emphasis on human MSCs and stem cell-mediated bone regeneration (chapter 2), NSCs and role of sex in neuronal development and stem cell-mediated neuroprotection (chapter 3) as well as on stem cells originating from the neural crest (NC) and sex differences in neurocristopathies (chapter 4). As an outlook, we will emphasize the potential role of sex differences in disease phenotypes as well as in the behavior of neural crest-derived stem cells (NCSCs), MSCs and NSCs.

The impact of sex in mesenchymal stem cell-mediated bone regeneration

Bone regeneration is a highly orchestrated process crucial for endogenous healing procedures after accidents, infections, or tumor therapy. Emphasizing the impact of sex-specific differences in bone repair, a prospective study including 1133 patients with intracapsular fractures of the femoral neck treated by internal fixation evidenced an increased risk for intracapsular hip fractures developing nonunion in females compared to their male counterparts [31]. Female sex can thus be

considered as a major risk factor for compromised bone healing [31,32] and is also associated with decreased osseointegration of implants [33]. Closely associated with impaired fracture healing, the prevalence of osteoporosis is known to be much higher in postmenopausal women than in older men, suggesting a positive correlation to estrogen deficiency in females [34,35]. Since native bone harbors too few osteoblasts to enable a fast recovery of acutely injured bone tissue on cellular level, stem cells need to give rise to new osteoblasts directly upon injury [36]. Osteogenic stem cell compartments such as MSCs are thus suggested to be critical for the differences in healing capacity observed between the sexes. Accordingly, estrogen was shown to modulate the osteogenic activity of mouse MSCs in a mouse model of osteoporosis [17]. Notably, sex-dependent changes in behavior of MSCs and other osteogenic stem cell compartments were also widely reported to occur in a nonhormonal driven manner [32,37,38]. Strube and coworkers reported a sex-specific compromised bone healing in female rats, which was directly associated with a decreased mesenchymal stem cell quantity. In particular, a 1.5-mm osteotomy gap in the femora of rats stabilized by an external fixator led to compromised mechanical competence of the callus as well as reduced callus size and mineralization in females compared to males. Although functional characteristics of male and female MSCs were similar, female bone marrow contained significantly fewer MSCs [32]. Sex-dependent differences were also reported in terms of osteogenic differentiation behavior of stem cells. Corsi *et al.* [38] showed a significantly greater ALP activity, expression of osteogenic genes, and mineralization after osteogenic differentiation of male mouse skeletal MDSCs compared to female cells. Transplanted male stem cells further revealed a more consistent and denser ectopic bone formation than female cells *in vivo*. In line with these findings, human stem cells derived from male adipose tissue (ASCs), namely the superficial or deep layer of abdominoplasty specimen, were shown to more efficiently undergo osteogenic differentiation in comparison to their female counterparts. Interestingly, while no significant difference in the degree of osteogenic differentiation was observable between ASCs from both female depots, ASCs from the male superficial depot differentiated faster and more efficiently compared to male deep layer ASCs [37]. These findings suggest sex-specific differences between the osteogenic activities of stem cell compartments also to occur in dependence to the anatomical region [37]. In line with the sex-specific decline of osteogenic differentiation capability in human ASCs, Muschler *et al.* [39] reported the amount of osteoblast progenitors in bone aspirates harvested bilaterally from the anterior iliac crest to significantly decrease with age of female but not male patients.

On mechanistical level, sex dimorphisms were also observed to be present in activated mouse mesenchymal stem cell function. In comparison with male MSCs, exposure of female MSCs to LPS, hypoxia, or H_2O_2 resulted in increased production of VEGF, while the expression of TNF-α was significantly reduced even after stimulation with LPS or hypoxia [40]. Accordingly, we also recently observed sex-specific expression patterns in differentiated human NCSCs regarding the genes SOD2, IGF2, and PKA cat alpha [41]. Like TNF-α, SOD2, IGF2, and PKA cat alpha are commonly known NF-κB target genes, suggesting a role of NF-kB in orchestrating sex-specific differences in stem cell behavior on a molecular level. A mouse paralog of the human NF-κB target gene SERPINA3 encoding the alpha 1ACT [42], the serine protease inhibitor Serpina3n was found to be dominantly expressed in female mouse osteoblasts. Female-dependent suppression of the osteoblast phenotype as for instance observed in terms of a significantly decreased activity of ALP in differentiated female osteoblasts compared to their male counterparts was dependent on the presence of Serpina3n. In particular, RNAi targeting of Serpina3n led to a significantly enhanced expression of ALP, Osteocalcin, and collagen type I, while its overexpression significantly suppressed both the mRNA levels of Osterix, ALP, Osteocalcin, and Col1a1 as well as mineralization [43]. Notably, crucial osteogenic transcription factors like Osterix or hydroxyl apatite-binding proteins such as Osteopontin are also well-known target genes of NF-κB [44,45].

In summary, female individuals seem to have lower quantities of osteogenic stem cell compartments in comparison with males [32,39], which is accompanied by a reduced differentiation potential into osteogenic derivates [37,38] (see Fig. 1 for overview). These differences may at least in part account for the impaired fracture healing and increased prevalence of osteoporosis observed in female individuals [31,32,34]. Although underlying molecular mechanisms still remain unclear, increasing evidences suggest a role of NF-κB in orchestrating sex-specific differences in osteogenic stem cell behavior.

The role of sex in neuronal development and neural stem cell-mediated neuroprotection

In the commonly accepted old model of sexual differentiation of the brain, genetic sex causes gonad

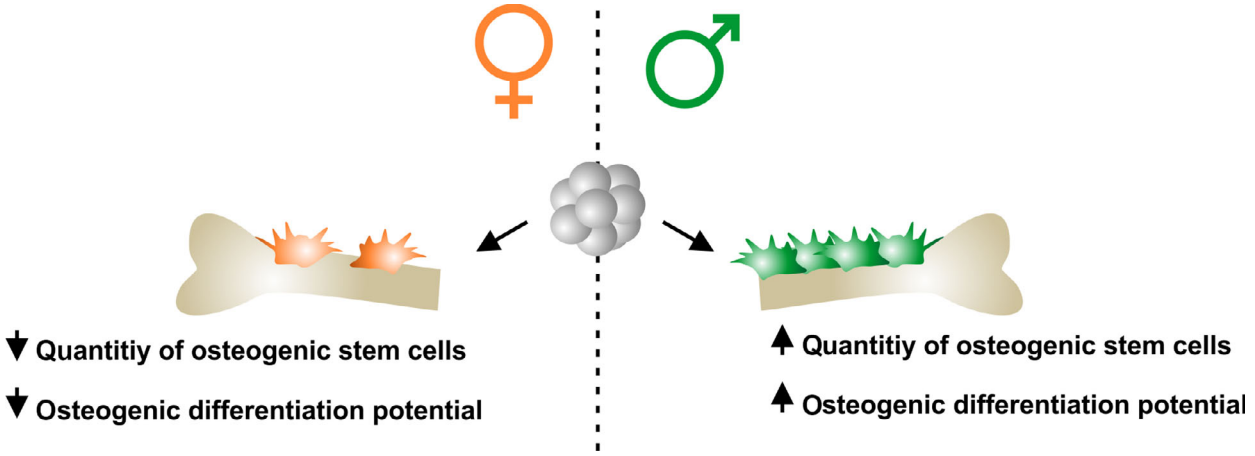

Fig. 1. Schematic view on sex-specific differences in osteogenic stem cells. Female individuals seem to have lower quantities of compartments in comparison to males, which is accompanied by a reduced differentiation potential into osteogenic derivates.

differentiation into female or male gonads. Gonadal hormones produced by the female or male gonads in turn mediate functional sex differences. However, inherent genetic differences and cell-intrinsic cellular mechanisms are increasingly noticed to account for sex-specific differences found in the brain in a hormonal-independent manner [46]. A sex-dependent regulation of hippocampal neurogenesis mediated by circulating sex hormones was described by Hillerer *et al.* [47]. Accordingly, males and females have different capacities to build new hippocampal neurons. Females express a greater number of mossy fiber synapses in the CA3 region [48] and produce more granule cells, but also have a higher degeneration rate than males [49]. These sexual dimorphisms may be due to different levels of circulating sex hormones. For example, ovarian hormones have been shown to be important for the regulation of hippocampal neurogenesis. Hillerer *et al.* [47] published that under baseline conditions the number of immature neurons in the dentate gyrus was higher in males compared to females. Chronic stress (2 h of restraint stress for 12 days) resulted in several sex-specific alterations. In males, granule cell proliferation decreased and an increase of quiescent stem cells was reported, while no changes were observed in females despite a decrease in cell survival.

Interestingly, sex-specific differences are found in development of the peripheral nervous system (PNS), which mostly develops from migrating NC cells [50] (see also chapter 4). McKey *et al.* [51] reported that NC cells colonize the interior mouse ovary at E16.5 and differentiate into neurons and glia. These NC-derived neuronal derivates were further shown to form the entire ovarian neural network. Notably, NC cells did not invade and innervate the testis, suggesting a sexual dimorphism mediated by repulsive cues in the male pathway [51]. In accordance with the major role of NC cells in development of the PNS, neurocristopathies of the nervous system like Hirschsprung disease (HD) or Waardenburg syndrome show sex-specific differences in terms of prevalence in males, as discussed in detail in chapter 4.

As introduced above, differences between the sexes in neuronal development can be also directly observed on cellular level prior to gonadal-derived hormonal surges. Bramble *et al.* [29,30] revealed 103 differentially expressed transcripts between male and female murine embryonic neural stem cells using global gene expression analysis. Transcriptional sex differences were mostly involved in cellular replication, indicating a differential regulation of proliferative and stemness states between male and female embryonic NSCs during development. Du and coworkers showed that male rat embryonic neurons isolated before gonad differentiation or hormonal stimulation were more sensitive to nitrosative stress and excitotoxicity compared to their female counterparts [52]. While female embryonic neurons were found to be more sensitive to etoposide- and staurosporine-induced apoptosis, male embryonic neurons showed an incapacity to maintain intracellular levels of reduced glutathione [52]. These promising observations regarding sex-specific differences can be also extended to the human system. During dopaminergic differentiation of human adult NC-derived nasal stem cells [inferior turbinate stem cells (ITSCs)], we discovered a significant higher maturation of female dopaminergic neurons in comparison to their male counterparts. This was detected by analysis of neurite outgrowth, arborization, and higher expression of

tyrosine hydroxylase. After treatment with 6-OH dopamine, male neurons showed a higher death rate compared to their female counterparts (B. Kaltschmidt & L. M. Ruiz-Perera, unpublished) (see Fig. 2 for overview). In this line, sex and gender differences were reported with a men to women ratio of approximately 2 : 1 in Parkinson's disease [53]. Accordingly, sex differences were observed in other neurodegenerative diseases such as Alzheimer's disease [6], but also in ischemic stroke [54] and neurodegeneration [7]. Since this matter is directly associated with mechanisms of stem cell-mediated neuroprotection, we will focus on this issue in the following.

Recently, we analyzed NF-κB-p65 mediated sex-specific effects in neuroprotection of adult NCSCs from the inferior turbinate (ITSCs, see also chapter 4) [41]. After differentiation of ITSCs into MAP2/Neurofilament/Synaptophysin/vGlut2-positive glutamatergic neurons, we applied H_2O_2 to induce oxidative stress. Surprisingly, female neurons showed a strong increase in neuronal cell death (about 50%) in comparison with their male counterparts (25%) (see Fig. 2 for overview). Activation of NF-κB by the proinflammatory cytokine TNF-α-dependent stimulation resulted in significant neuroprotection against oxidative stress-induced cell death in both sexes. While female neurons upregulated the NF-κB target genes SOD2 and IGF2 to induce neuroprotection, male neurons showed elevated expression of the neuroprotective NF-κB target gene PKA cat alpha [41]. SOD2 is well known for protecting cells against oxidative stress resulting in longevity [55]. IGF-2 is known to promote synapse formation and spine maturation [56], and it has antioxidant, neuroprotective effects against oxidative damage [57,58]. Taken together, we could show that NF-κB p65 is a key player in neuroprotection of human glutamatergic NC-derived neurons, although the protective gene expression program beneath is sexually dimorphic.

Next to oxidative stress, data from Wilhelm and coworkers suggested a sex-specific effect in ethanol abuse, leading to addiction. In mouse models analyzing both sexes, it was shown that female animals were more sensitive to ethanol, corresponding to higher expression of selected genes [59]. Moreover, after alcohol withdrawal, Wilhelm and co-authors could demonstrate that the transcription factor NF-κB is a central node (hub) in female as well as in male transcriptional gene networks. Nevertheless, none of the NF-κB-connected genes were regulated in common between the sexes,

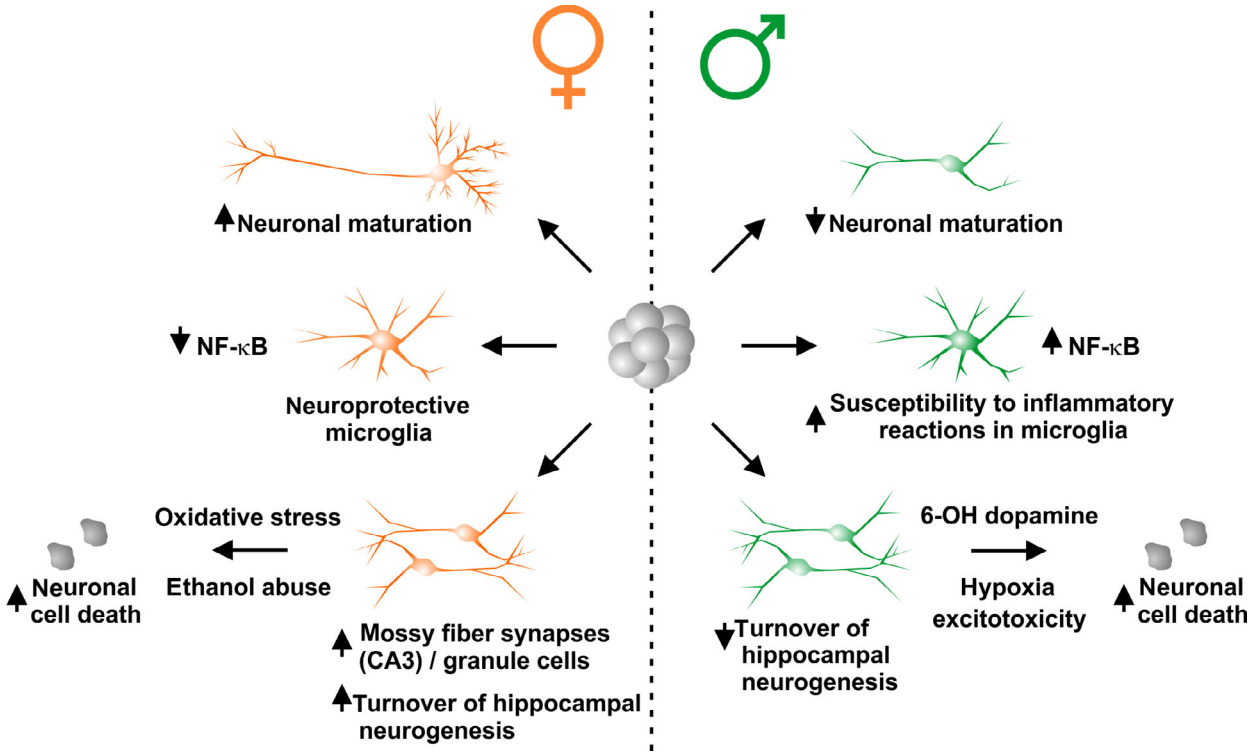

Fig. 2. Schematic view on sex-dependent differences occurring in NSC-driven neuronal development and stem cell-mediated neuroprotection.

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indicating a clear sexually dimorphic signaling pattern. In particular, females showed an upregulation of TNF-α, IL-6, CCL11 (Eotaxin) and CCL5, cytokines, and chemokines, which regulate proinflammatory responses. These findings are in accordance with proinflammatory toxicity and increased neuronal death observed in females, and might be associated with neurodegeneration in humans [60]. In contrast, males depicted a profound downregulation of chemokines such as CCL28, CCL5, and the receptor CXCR5. Overall, an immune suppression and a relative neuroprotection were observed in males. In summary, these studies demonstrated that sex has a very strong influence on the early response to chronic ethanol effects (see Fig. 2 for overview) and during the immediate withdrawal period.

In terms of sex-specific differences in neuronal survival, Wojcik and coworkers described a mouse model with a knockout of brain-specific angiogenesis inhibitor I-associated protein 3 (Baiap3) [61]. Baiap3 is a member of the mammalian uncoordinated 13 protein family of synaptic regulators of neurotransmitter exocytosis. This protein is highly expressed in amygdalae, hypothalamus, and periaqueductal gray brain regions relevant for emotionality and drug dependence. Knockout of Baiap3 resulted in increased latency to reach walls in open field and higher freezing percentage. Surprisingly, the penetrance of this phenotype was observed to be sex-specific with an increased anxiety in females with much lower latency in the open field test and reduced visits to the center in female KO mice [61]. Furthermore, male diazepam-treated Baiap3 KO mice showed significantly faster improvement in the rotarod test, consistent with a more rapid development of tolerance to diazepam. In addition, an increased anxiety was observed in both sexes, with a more pronounced effect in female KO mice. Finally, human BAIAP3 alleles were discovered that are associated with anxiety in women and benzodiazepine use disorder in men [61].

Using cultivated hippocampal neurons derived from Wistar rats, profound survival differences between different sexes were recently shown after hypoxia induction [62]. Under normoxic conditions, neurons from males survived slightly better than their female counterparts. However, under hypoxia, cell death in male neurons was increased in contrast to females (see Fig. 2 for overview). Moreover, cell death was more prominent in female neurons derived from pubs, which had a systemic treatment with testosterone. Interestingly, treatment of male hypoxic hippocampal cultures with 17-ß-estradiol acted neuroprotective with cell death levels similar to controls, suggesting a neuroprotective role of estrogen during hypoxia. Hypoxia was induced for 15 h in an incubator purged with 95% N_2 and 5% CO_2 and further led to increased expression of estrogen receptor beta only in male cultures, whereas aromatase was found to be expressed about one-third higher in female neurons [62]. A similar vulnerability of male neurons to the neurotoxic effect of dopamine was published by Lieb and coworkers [63]. Interestingly, Villa and coworkers recently observed significant differences in the transcriptome of microglia from male and female adult mice, particularly regarding inflammatory target genes of NF-κB [64]. Seventy-nine percentage of 95 inflammatory NF-κB target genes showed increased expression in male microglia compared to their female counterparts, demonstrating a proinflammatory transcriptional activity of NF-κB and greater susceptibility to inflammatory reactions in male microglia. Accordingly, female microglia showed a neuroprotective phenotype, which was still present after transfer into mail brains and protected male brains from ischemic stroke [64] (see Fig. 2 for overview). The maintenance of sex-specific features of microglia after transplantation into the brain of the opposite sex as well as during *in vitro* culture indicated a cell-intrinsic mechanism independent from hormonal cues to account for the observed sex-specific differences.

In summary, sex-dependent differences exist both in neuronal development and in stem cell-mediated neuroprotection in a cell-intrinsic manner, which is increasingly noticed as additional crucial mechanism next to hormonal cues. While female neurons show a higher susceptibility to increased proinflammatory toxicity and neuronal death compared to male neurons, for instance in terms of oxidative stress [41] or ethanol abuse [59], female microglia are neuroprotective in contrast to their male counterparts [64]. Females also show elevated levels of granule cells and a greater number of mossy fiber synapses in the CA3 region of the hippocampus, but also have a higher degeneration rate than males during hippocampal neurogenesis [48,49]. On the contrary, male neurons show increased susceptibility to cell death after exposure to hypoxia [62] and 6-OH dopamine [63] (B. Kaltschmidt & L. M. Ruiz-Perera, unpublished). On mechanistical level, NF-κB seems to be a key player in neuroprotection of both sexes, although the protective gene expression program beneath is sexually dimorphic.

Adult neural crest-derived stem cells and sex-specific differences in occurrence and severity of diseases related to neural crest development

The NC was identified as an embryonic structure by Wilhelm His in 1868, namely as the 'Zwischenstrang'

(intermediate chord) in the developing chicken embryo [65]. Wilhelm His described the NC as a transient cell population arising during neurulation between the neural tube and the future ectoderm [65] reviewed in Ref. [66]. After neurulation of the vertebrate embryo, NC cells undergo epithelial to mesenchymal transition and migrate into a broad range of tissues of the developing embryo, where they directly contribute to embryonic development [67] reviewed in [66,68,69]. Here, NC cells particularly differentiate to ectodermal and mesodermal cell types, like cardiac NC cells contributing to the formation of the heart, sacral, and vagal NC cells participating in development of enteric neurons and glia, or trunk NC cells giving rise to sensory and sympathetic ganglia [68,70,71]. Cranial NC cells form vital craniofacial cell types and structures of the head region, such as bone tissue or peripheral nerves [66]. Next to directly giving rise to distinct cell types, NC cells were also reported to guide patterning and differentiation of their target tissues during embryogenesis, for instance in vertebrate stomach development [72] or during myogenesis [73].

Neural crest stem cells not only directly contribute to embryonic development, but are commonly known to reside within the organism until and during adulthood as dormant stem cells termed NCSCs. Human NCSCs can be isolated from various adult tissues, such as skin [74,75], periodontium [76], or the nasal cavity [77–79]. Common characteristics of isolated NCSCs particularly include their easy accessibility, expression of Nestin, $p75^{NTR}$, and the NC markers SLUG, SNAIL, TWIST, SOX9, and SOX10 as well as the ability to self-renew and formation of neurospheres *in vitro* (reviewed in [66]. Next to these characteristics, adult NCSCs display a broad differentiation capability into mesodermal and ectodermal cell types [74,77,80–83]. We previously reported the presence of a particularly interesting population of NCSCs within the respiratory epithelium of the inferior turbinate in the human nasal cavity [78]. We demonstrated in several studies that such ITSCs are able to differentiate into a wide range of mesodermal and ectodermal cell types [78,81,84]. Namely, ITSCs gave efficiently rise to glutamatergic [41] and dopaminergic neurons [82] but also to mesodermal derivates like chondrocytes, adipocytes, or osteocytes [80,81,85,86]. Due to their extraordinary broad differentiation potential, NCSCs and particularly ITSCs seem an ideal model system for understanding potential sex-specific differences in endogenous differentiation and regeneration processes. In this regard, we recently observed sex-specific differences in neuroprotection of glutamatergic neurons differentiated from ITSCs, which was mediated by NF-κB-p65. Here, TNF-α-dependent activation of NF-κB-p65 was accompanied by significant neuroprotection against oxidative stress-induced neuronal death, which was surprisingly higher in neurons from female donors compared to their male counterparts [41] (see also chapter 3 and Fig. 2). These observations strengthen our hypothesis of sex-specific differences to occur in the behavior of adult NCSCs.

Their crucial contribution to development directly associates dysfunction of NC stem cells to certain disease phenotypes, so-called neurocristopathies, which were introduced as a summarizing concept of diseases arising in terms of NC maldevelopment by Robert Bolande, [87]. Bolande discussed increasing evidences that a number of dysgenetic and neoplastic conditions show a common distinct patho-genetical origin in the malformation of the NC or the dysfunction of embryonic NC stem cells or its derivatives. The diseases are categorized into four categories: tumors, tumor syndromes, malformations, and all other neurocristopathies (see Fig. 3 for overview). NC-derived tumors particularly include melanoma, neuroblastoma, neurofibroma, medullary thyroid cancers, and pheochromocytoma. Further malformations include Tietz albinism-deafness syndrome, cleft lip or palate, heart malformations, and craniofacial defects [66,87]. These craniofacial defects particularly include the Saethre–Chotzen syndrome, a failure of neural tube closure related to heterozygous TWIST1 mutation leading to craniosynostosis and facial dysmorphism [88] (see also OMIM entry 101400). While inner ear neurocristopathies arise from melanocyte defects, craniofacial neurocristopathies of the outer and middle ear can be caused by NC developmental defects of bone and cartilage [89]. For instance, mutations in TFAP2A lead to branchiooculofacial syndrome, where anomalies of the external and middle ear frequently cause conductive hearing loss [90,91] (see also OMIM entry 113620). The Treacher Collins–Franceschetti syndrome is also associated with NC maldevelopment, where a TCOF1 mutation leads to NC apoptosis and causes among others deformity of the ears, conductive hearing loss, and cleft palate [92] (see also OMIM entry 154500). Additional factors involved in neurocristopathies of the outer and middle ear are endothelin or BMP signaling [89]. Mutations or loss in Pax9 is also associated with absent teeth, so-called selective tooth agenesis [93] (see also OMIM entry 604625). For further information regarding craniofacial defects related to NC malformation, see [66,94]. Commonly known NC-related diseases further include Hirschsprung's disease and the associated Waardenburg syndrome as well as

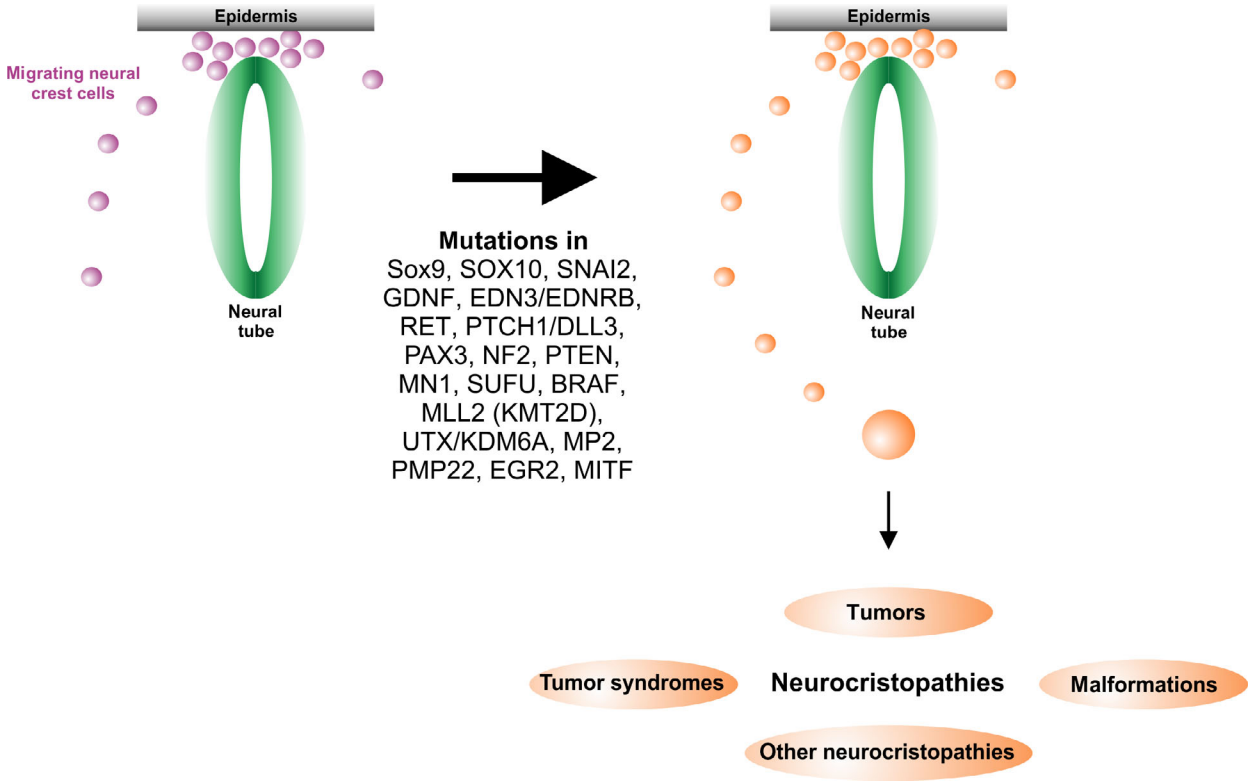

Fig. 3. Schematic view on the genetic causes of neurocristopathies, a summarizing concept of diseases arising in terms of NC maldevelopment. For references regarding the listed mutations, see also Table 1.

Charcot–Marie–Tooth disease. However, the potential occurrence and impact of sex-specific differences in prevalence and severity of such neurocristopathies disease are still underestimated. In Table 1, we provide a detailed summary of neurocristopathies showing such sex-specific differences in prevalence together with respective affected cell types, afflicted genes (see also Figs 3–4), and frequencies of occurrence (Table 1). Sex-specific neurocristopathies include Hirschsprung's disease, Waardenburg syndrome, Meningioma, Melanoma, Kabuki syndrome, Kabuki syndrome-2, Charcot–Marie–Tooth disease, and Tietz albinism-deafness syndrome and will be also discussed in the following.

Hirschsprung disease is a motor disorder of the gut, where nerves are absent in parts of the intestine [95]. The incidence is approximately 1 in 5000 live births. HD is caused by the failure of NC cells (precursors of enteric ganglion cells) to migrate completely during intestinal development during fetal life. The resulting aganglionic segment of the colon fails to relax, causing a functional obstruction. Several other neuropathies are associated with HD, such as Waardenburg syndrome, which includes pigmentation defects and deafness. Most HD patients have mutations in the tyrosine kinase receptor RET in coding or upstream sequences. RET is the receptor of glial cell line-derived neurotrophic factor (GDNF) and is required for the enteric NC migration. Other involved yet less affected genes include GDNF, SOX10, endothelin 3 (EDN3), or endothelin receptor (EDNRB) (see Table 1 for overview). A rare defect of HD is a mutation of L1CAM in newborn males, resulting in hydrocephalus or agenesis of the corpus callosum. As introduced above, the most accepted theory of the cause of HD is that there is a defect in the craniocaudal migration of neuroblasts originating from the NC, a process that begins at 4 weeks of gestation and ends at week 7 with the arrival of NC-derived cells at the distal end of the colon. RET gene mutations were found in NC-derived cells of HD patients, leading to large deletions and other loss of function mutations. In short segment HD, where the aganglionic segment does not extend beyond the upper sigmoid (80% of the cases) a strong sex bias exists with more affected males (sex ration four male/one female). In this line, it was recently shown that mosaicism of RET mutations in HD is still underestimated [96]. The associated Waardenburg

Table 1. Diseases related to the NC (neurocristopathies) and their sex-specific prevalence. The OMIM ID corresponds to the respective afflicted genes stated on the left.

Name	Defect	Afflicted genes	OMIM ID	Frequency (birth)	Sex-specific prevalence
Hirschsprung's disease	Parasympathetic nervous system ganglia of enteric plexus	RETGDNFSOX10EDN3/ EDNRBPTCH1/DLL3	142623 (Ref. [107])	1 : 5000	Male
Waardenburg syndrome	Neurons of enteric plexus	PAX3SNAI2SOX10	193500193510	1 : 40 000	Male
Meningioma	Arachnoid cap cells (meningothelial, telencephalic lepto-meninges from NC)	NF2PTENMN1 caused by SUFU mutation	607174	1-9 : 100 000; adult onset: 34% of all brain tumors1 : 33 in women	Twice more common in women
Melanoma	Melanocytes	Sox9 (weak or absent expr.)BRAF activation (40–60%)	155600	Unknown	Unknown; mortality: 2/3 in men
Kabuki syndromeKabuki syndrome-2	Craniofacial defects, cardiac defects, postnatal growth retardation	MLL2 (KMT2D) UTX/ KDM6A	147920300867	1–9 : 100 000	X-linked female
Charcot–Marie–Tooth disease	Schwann cells	MP2PMP22EGR2	118300	1 : 2500	Sometimes X-linked
Tietz albinism-deafness syndrome	Melanocytes, sensorineural hearing loss	MITF	156845	1 : 1 000 000	X-linked, only male phenotype

syndrome is also known to have a sex-specific prevalence in male patients (Table 1).

In addition to sex-specific differences in Hirschsprung's disease and Waardenburg syndrome, NC-derived tumors show sex-specific differences. Here, meningiomas, which are tumors of NC origin developing from meninges, show more than twofold frequency in women in the age of 30–70 years in comparison with males. In contrast, malignomas are more frequent in males. Hormone replacement therapy in women is suggested to increase meningiomas [97]. Accordingly, long-term hormone replacement therapy,

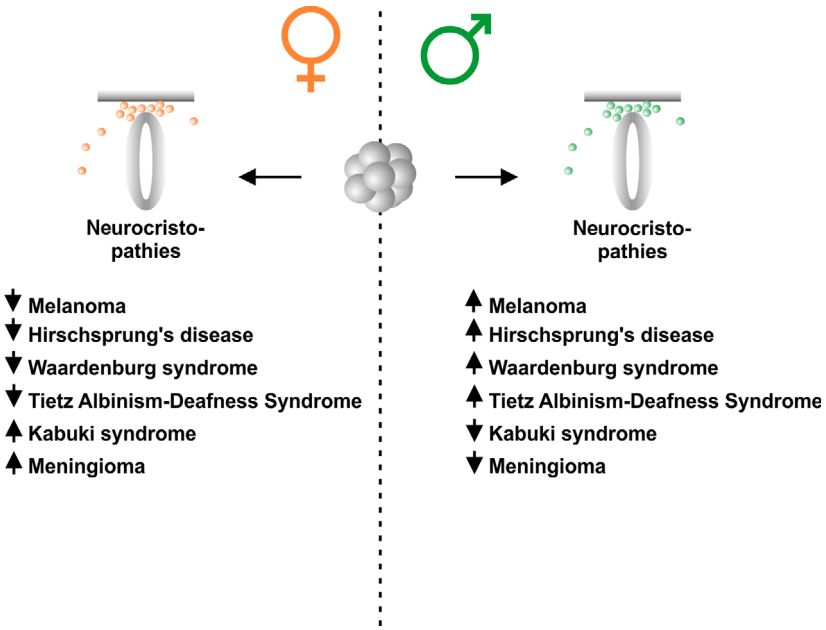

Fig. 4. Sex-specific occurence and prevalence of neurocristopathies. For detailed information, see also Table 1.

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particularly combined estrogen–progestogen therapy, was also linked to an increased risk of developing meningioma in females [98].

Next to the sex-specific occurrence of NC-related tumors, Kabuki syndrome is known as a X-linked disease (Table 1). Kabuki syndrome is a craniofacial disorder caused by mutations in an X-linked histone H3 lysine 27 demethylase (UTX/KDM6A) or a H3 lysine methylase (KMT2D). Features of Kabuki syndrome such as craniofacial defects, cardiac defects, and postnatal growth retardation could be reproduced in mice with UTX (Ubiquitously transcribed tetratricopeptide repeat, X chromosome deletion) in NC [99]. NC specific deletion was achieved by the use of Wnt1-Cre line. The observed defects might not depend on the enzymatic demethylase activity. Female UTX KO mice have an enhanced phenotypic severity over male, presumably due to partial rescue by UTY, a methylase dead homolog of UTX on the Y chromosome. In addition to the Kabuki syndrome, Charcot–Marie–Tooth disease, and Tietz albinism-deafness syndrome are also known to be X-linked. Accordingly, the Tietz albinism-deafness syndrome shows a phenotype only in male individuals (Table 1). In summary, these observations emphasize the sex specificity of a broad range of neurocristopathies, which seem to be either at least directly linked to the X chromosome or even have a higher prevalence in male individuals in most cases. The here discussed sex-specific differences in neurocristopathies and the direct involvement of mutations in key markers for NCSCs further suggest sex-specific differences to occur in NCSC-behavior, which is in accordance with our very recent observations in adult NCSCs [41].

Notably, structural and functional sex differences in brain areas during development are also closely related to a broad range of commonly known sex-dependent differences in behavior and neuropsychiatric disorders [100]. Here, male individuals seem to have a prevalence to disorders with distinct developmental origin such as autism or schizophrenia, while females are more likely to be diagnosed with diseases occurring after the onset of adolescence like depression and anxiety disorders [100,101]. Linking these observations to stem cell behavior, proliferation of neural stem cells was described to be decreased in human brain specimens from patients with schizophrenia but not in those from patients with depression [102]. On genetic level, deletion of PTEN, which germline mutations are found in a subset of children suffering from autism spectrum disorder, was further described to result in an initial increase of self-renewal of NSCs in the dentate gyrus followed by a shift from neuronal to astrocytic differentiation [103,104]. As we reviewed in chapter 3, mechanisms of stem cell-mediated neuroprotection are also dependent on the sex of the individual with direct

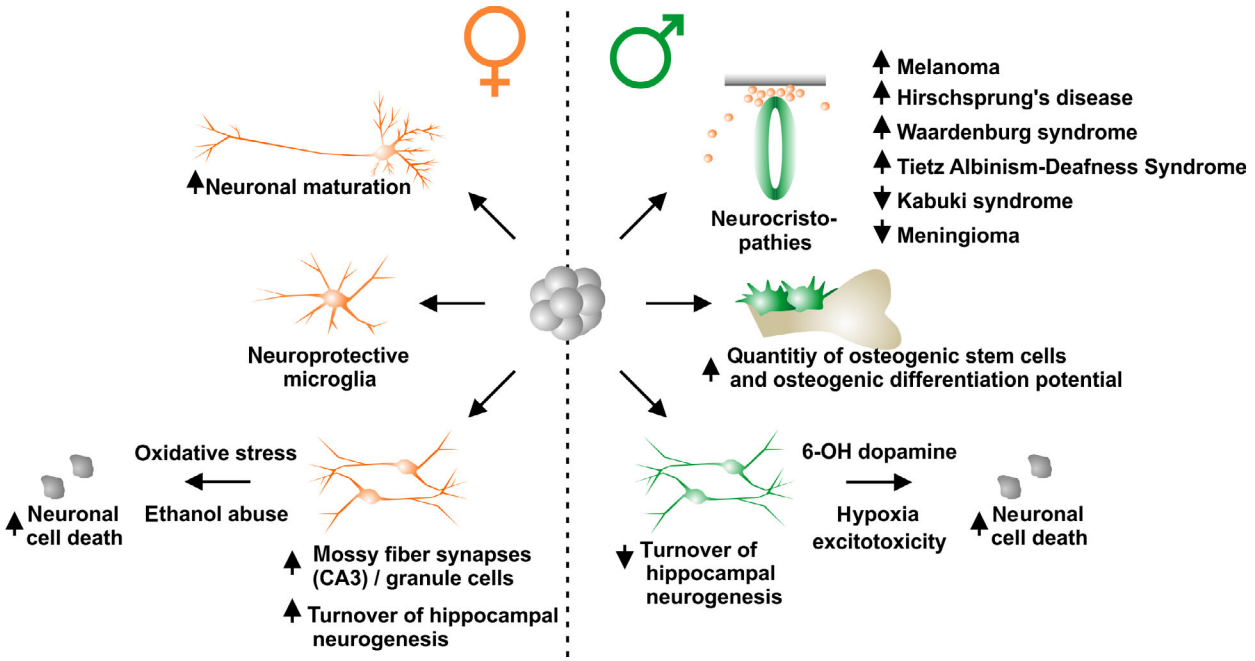

Fig. 5. Schematic summary of sex-specific differences of stem cell behavior in neurogenesis, neuronal maturation, neuroprotection, osteogenic differentiation, and neurocristopathies.

implication for sex differences reported in Parkinson′s disease [53], Alzheimer's disease [6], ischemic stroke [54], and neurodegeneration [7].

Although some rare disorders of bone development are genetically linked to the X chromosome, like osteopetrosis or X-linked spondyloepiphyseal dysplasia [105,106], a broad range of bone diseases like osteoarthritis and osteoporosis are predominantly occurring in female individuals compared to their male counterparts. These diseases may at least in part be driven by the differences between male and female MSCs concerning their amounts and efficiencies in undergoing osteogenic differentiation [37,39] (see also Chapter 2 and Fig. 1).

Outlook

In summary, the present review emphasizes sex-specific differences in the behavior of mammalian stem cells, which are graphically summarized in Fig. 5. In terms of MSCs, males show an increased quantity of osteogenic stem cell compartments accompanied by elevated potentials of osteogenic differentiation compared to females (see chapter 2 and Figs 1, 5 for overview). While female individuals show increased maturation of neuronal differentiated stem cells as well as increased numbers of mossy fiber synapses and granule cells in the hippocampus, they also exhibit an increased turnover of hippocampal neurogenesis and elevated neuronal cell death after alcohol abuse or oxidative stress compared to males (see chapter 3 and Figs 2, 5 for overview). Neuroprotective microglia are further only observed in females in contrast to their male counterparts (see chapter 3 and Figs 2, 5 for overview). Turnover of male hippocampal neurogenesis was observed to be reduced, although male neurons differentiated from stem cells are more susceptible to 6-OH dopamine and hypoxia than their female counterparts (see chapter 3 and Figs 2, 5 for overview). With NC-related neurocristopathies being at least directly linked to the X chromosome or even having a higher prevalence in male individuals in most cases, the here discussed observations suggest sex-specific differences to occur in NCSCs and influence their behavior (see chapter 4 and Figs 3–5 for overview).

The here discussed findings suggest that sex-related differences in the health of the stem cell compartment could at least partially explain different rates of aging and diseases observed between female and male individuals. Interestingly, such sex differences may also account for the broad heterogeneity commonly observed in phenotype and performance of human stem cells [14]. Despite these observations, most studies of the brain [46] or bone diseases as well as those focusing on the respective stem cell niches or isolated stem cell populations focus on one sex, which is usually male. In addition, a broad range of studies still lacks reporting sex of animals or human donors/patients applied in the experimental setup. In accordance with the observations made by McCarthy in 2011 in terms of the brain [46], the still widespread assumption that the influence of sex may be negligible in the field of stem cell research is clearly misleading for research approaches and thereby delays or even prevents progress. From a clinical perspective, the unilateral study of only one sex, namely the male one, leads to an only partial understanding of disease mechanisms and questions correct medications and therapy for female individuals. With increasing evidence pointing toward sex-specific differences in neurocristopathies as well as in degenerative diseases such Parkinson's disease or osteoporosis, differences between male and female should be more accredited and further investigated in basic research but also in a therapeutic context. Here, particularly the sex-dependent differences in behavior of stem cells discussed here may become apparent as a crucial parameter for potential future treatment strategies.

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