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REVIEW

Role of *Hox* genes in stem cell differentiation

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Abstract

Hox genes are an evolutionary highly conserved gene family. They determine the anterior-posterior body axis in bilateral organisms and influence the developmental fate of cells. Embryonic stem cells are usually devoid of any *Hox* gene expression, but these transcription factors are activated in varying spatial and temporal patterns defining the development of various body regions. In the adult body, *Hox* genes are among others responsible for driving the differentiation of tissue stem cells towards their respective lineages in order to repair and maintain the correct function of tissues and organs. Due to their involvement in the embryonic and adult body, they have been suggested to be useable for improving stem cell differentiations *in vitro* and *in vivo*. In many studies *Hox* genes have been found as driving factors in stem cell differentiation towards adipogenesis, in lineages involved in bone and joint formation, mainly chondrogenesis and osteogenesis, in cardiovascular lineages including endothelial and smooth muscle cell differentiations, and in neurogenesis. As life expectancy is rising, the demand for tissue reconstruction continues to increase. Stem cells have become an increasingly popular choice for creating therapies in regenerative medicine due to their self-renewal and differentiation potential. Especially mesenchymal stem cells are used more and more frequently due to their easy handling and accessibility, combined with a low tumorgenicity and little ethical concerns. This review therefore intends to summarize to date known correlations between natural *Hox* gene expression patterns in body tissues and during the differentiation of various stem cells towards their respective lineages with a major focus on mesenchymal stem cell differentiations. This overview shall help to understand the complex interactions of *Hox* genes and differentiation processes all over the body as well as *in vitro* for further improvement of stem cell treatments in future regenerative medicine approaches.

Key words: Genes; Homeobox; Stem cells; Asymmetric cell division; Mesenchymal stromal cells; Growth; Development; Regeneration; Patterning; Cell lineage

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Core tip: *Hox* genes are involved in embryonic development as well as in repair mechanisms in the adult body, thus

regulating cell fate. These genes have also been found to be driving factors in various stem cell differentiations *in vitro* and *in vivo* which makes them interesting tools for future improvements in stem cell therapies. Therefore, this review outlines the involvement of *Hox* genes in various stem cell differentiations with a major emphasis on mesenchymal stem cell differentiations.

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INTRODUCTION

The life expectancy of the overall population has increased in most countries, accompanied by an increase in injuries, diseases, and age related degenerative diseases. Thus, reconstructions of hard and soft tissues are an increasing challenge in regenerative medicine[1]. Stem cells could help to overcome these restrictions using their self-renewal and differentiation properties, with or without biomaterials or artificial scaffolds[1-3]. Numerous sources are known from which stem cells can be isolated, often with a varying differentiation potential[4]. Additionally, the commitment might depend on the body site from which the stem cells were isolated[4,5]. *In vivo*, stem cell fate is determined according to tissues and thus extracellular but also intracellular factors, among them p53, purinergic receptors, collagens, parathyroid hormone-related proteins, or homeobox (*Hox*) genes[6-9]. The later are the central players in determining regional specificity in the developing embryo and not astonishingly also in numerous differentiations[10].

The *Hox* gene family consists of 39 members, which are clustered in four paralogous groups and are numbered from 1-13 within the groups. During embryogenesis, *Hox* genes show an expression pattern which is specific for each body region, commonly referred to as the *HOX* code. They are an evolutionary highly conserved collection of genes binding to various DNA recognition sites and thus influencing a cell's developmental fate in a huge variety of species, while determining the anterior-posterior body axis[11] (Figure 1). The precise function of these genes is achieved by their specific temporal and spatial activation over the course of life. Due to this role in embryonic and adult developmental stages, *Hox* genes are expected to also have fundamental influences on the differentiation of various stem cells *in vivo* and *in vitro* towards their respective lineages. There have been studies which directly identified *Hox* gene involvement in stem cell differentiations, and others which suggest their role based on observed effects of *Hox* genes in *in vivo* or *in vitro* tissue development. It has been speculated that the code may contribute to maintaining the stem cell character in certain niches, since it is also regulated on an epigenetic level[12].

This review will outline the *Hox* profile of various stem cells, and discuss the wide range of findings concerning *Hox* genes in stem cell differentiations gathered over the last decade. The nomenclature used for *Hox* genes in this study is according to the current regulations, meaning that *Hox* genes related to findings in mice and rats are written with the first letter capitalized, whereas the following are in small capitals (*e.g., Hoxc8*). For findings in human and chicken, all letters are capitalized (*e.g., HOXC8*). Understanding the principle behind the role of *Hox* genes in differentiations and development may provide improved possibilities to alter stem cell fate *in vitro* and *in vivo*, and therefore might result in new or improved approaches in regenerative medicine.

Therapeutic approaches are more and more based on the use of mesenchymal stem cells (MSCs), due to their easy accessibility and handling compared to other kinds of stem cells. Hence, we put specific emphasis on the differentiation lineages of MSCs within this review.

HOX TRANSCRIPTION FACTORS IN STEM CELLS

Embryonic stem cells

Embryonic stem cells (ESCs) can be gained from the inner cell mass of the blastocyst and are therefore derived from an early stage embryo[13,14]. They are characterized by various markers, having the most significant surface markers SSEA-1, SSEA-3, SSEA-4 as well as TRA-1-60 and TRA-1-81[15]. Additionally ESCs are positive for the transcription factors NANOG and OCT3/4[16,17]. Their pluripotency, the potential to differentiate into each cell lineage and all types of cells of the body, and the proliferation rate causes huge interest in this stem cell type[18,19]. An active repression of *Hox* clusters has been demonstrated in various studies. On the other hand *Hox* genes are activated when differentiation processes take place[20]. However, it is not known which *Hox* code exactly commits these cells into the different lineages, yet[20].

Hematopoietic stem cells

Hematopoietic stem cells (HSCs) are the best characterized type of adult stem cells[21]. They can be obtained from bone marrow, cytokine-mobilized peripheral blood, and cord blood[22]. HSCs are characterized with the antigen cell-surface markers CD34, C-KIT, TIE, and CD133/AC133 and they lack CD38, LIN, and CD45RA[23]. These stem cells differentiate into all kinds of blood cell lineages serving the reconstruction of the lympho-hematopoietic system[24]. In general, the *HOXA* cluster plays a role in hematopoiesis[25], but the expression of *HOXB4* which directs transition from the embryonic to the adult hematopoietic program is a major factor as

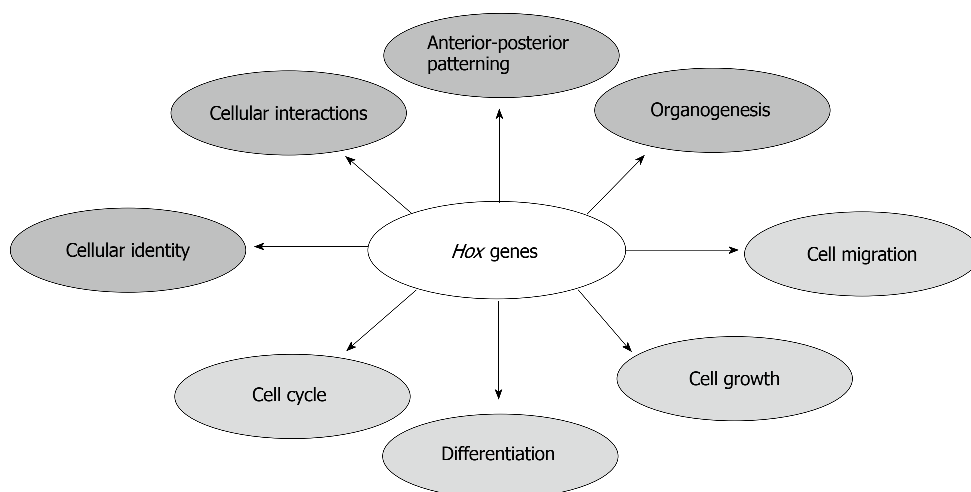

Figure 1 Roles of *Hox* genes in the organisms and the cell[135-140].

well[21].

Neural crest cells

Neural crest cells (NCCs) are multipotent cells exclusively present in vertebrates[26]. These cells are not only found in the early embryonic development but are also present in various neural crest-derived tissues in the fetal and adult organism[27]. NCCs are located in between the neural epithelium and epidermis in the neurulating vertebrate embryo[26]. After they have undergone epithelial-mesenchymal transition the NCCs migrate within the embryonic tissue to the respective location and undergo differentiation[28]. This stem cell type commits towards facial cartilage, bone and connective tissue as well as neural and glial cells of the peripheral nervous system. Additionally, they are able to differentiate into skin pigment cells, mesenchyme and in restricted locations into smooth muscle cells[29]. NCCs are characterized with the markers p75NTR, AP-2α and the transcription factors MSX1, MSCX2 and SLUG in the pre-migratory state[30]. In post-migratory state, embryonic and adult cell markers can be distinguished as follows: in the embryo, adult gut and bone marrow p75 is present, whereas SOX10 is found in the dorsal root ganglia of the embryo and in the adult bone marrow. As an additional marker NESTIN is suggested because it is present in the adult heart and embryonic boundary cap cells. Other markers are MUSASHI in the adult heart, SCA1 and CD34 in the adult cornea as well as SLUG and SNAIL in the adult bone marrow[26]. Usually there are no *Hox* genes expressed in NCCs[31]. However data has been found where *Hoxa2* and *Hoxd9* can be induced in mouse trunk neural crest cells by retinoic acid, a major factor triggering differentiation[32]. Additionally, *Hoxd3* has been shown to have an impact on the formation of somitic mesoderm-and neural crest-derived structures during mouse development[33].

MSCs

MSCs are multipotent adult stem cells which can be *in vitro* differentiated towards lineages of mesenchymal tissues[7,34,35], which is in line with their natural repair function in the body where they regenerate mesenchymal tissues like bone, cartilage, muscle ligament, tendon and adipose tissues[7,36]. In addition it has been described that these stem cells can also differentiate towards lineages of the other two germ layers, such as towards endothelial cells, hepatocytes[37], or neurons of the ectoderm[38]. MSCs are found in the stroma of various tissues or organs such as bone marrow, umbilical cord, wisdom teeth and adipose tissues[5,7,39]. Defined characteristics of these stem cells are the plastic adherence under standard culture conditions, the presence of the antigen surface markers CD73, CD90 and 105 and the absence of CD34, CD45, CD14 or CD11b, CD79α or CD19 and HLA-DR[40]. Since *Hox* genes are involved in organ formation and regeneration, it is not astonishing that the degree of *Hox* gene expression in various adult stem cell populations differs which enables the discrimination of adult stem cells by the respective *Hox* expression pattern. Cord blood-derived MSCs (CB-MSCs) and unrestricted somatic stem cells (USSCs) are gained from cord blood. Whereas CB-MSCs are positive for *HOXA9, HOXB7, HOXC10* and *HOXD6*, USSCs resembling the profile of typical ESCs are negative for *Hox* genes. In CB-MSCs and USSCs it has been demonstrated that this expression code is in line with the differentiation potential of stem cells: CB-MSCs can be differentiated towards the adipogenic lineage whereas USSCs generally can be committed towards all three germ layers and show nevertheless no adipogenic differentiation. *HOXB7* and *HOXD6* expression has also been shown in bone marrow-derived MSCs that generally have a similar expression profile as MSCs derived from adipose tissue. Amnion- and decidua-derived MSCs can be distinguished using *HOXC10* expression[41]. Taken together, a distinct expression of *HOXA5* and *A10, HOXB6, B7, HOXC4, C6, C8, C9* and *C10* as well as *HOXD3* and *D8* is observed in MSCs derived from different human sources[42]. In murine MSCs the expression of *Hoxb2, b5, b7* and *Hoxc4* has

Table 1 *Hox* gene expression in various kinds of stem cells

Type of stem cell	*Hox* expression	Role of *Hox*
HSC	*HOXA* cluster *HOXB4*	Hematopoiesis and its transition from embryonic to adult program
MSC	*HOXA5, 10* *HOXB6, 7* *HOXC4, 6, 8, 9, 10* *HOXD3* and *D8*	Degree of *Hox* expression pattern differs in the different sources and determines differentiation potential
ESC	None	Active repression of *Hox* genes until commitment starts
CSC	Various	Overexpression of *Hox* genes present in undifferentiated cells and down-regulation *Hox* genes present in differentiated cells
	HOXA9	Hematopoiesis
	HOXB7, C6, C8	Vascularization
	HOXA4, D10	Colon carcinoma
NCC	*HOXD3*	Formation of somitic mesoderm- and neural crest-derived structures
iPS	None	Active repression of *Hox* genes until commitment starts

HSC: Hematopoietic stem cells; MSC: Mesenchymal stem cells; ESC: Embryonic stem cells; CSC: Cancer stem cells; NCC: Neural crest cells; iPS: Induced pluripotent stem cells.

been shown, which regulate self-renewal and influence the differentiation of other stem cells[43].

Cancer stem cells

Cancer stem cells (CSCs) are cancer cells with the capability of both, self-renewal and differentiation, which are the typical characteristics of any stem cell[44]. These stem cells are multipotent, clonogenic, have the capability to induce new tumors and eventually create heterogeneous cell lineages in tumors[45]. Accordingly they are thought to be responsible for progression of cancer, cancer metastasis, and therapy resistance of cancers[46]. In addition, the recurrence and spreading of cancer might be due to CSCs that remain in residual tumors after surgery and treatment. It was suggested that a tumor contains different stem cell like populations due to reduced cell differentiation within a tumor and this heterogeneous population also includes CSCs[44,45]. DNA damages may render tissue stem cells to become CSCs on their way from a stem cell to a fully differentiated cell. However, instead of dying at the different levels of differentiation like normal tissue stem cells do these cancer precursors do not differentiate normally but accumulate forming tumors[47]. Conclusively, this population differs in marker expression and growth capacities[45]. In general CSCs can be characterized by the presence of the markers CD24, CD29, CD44, CD90, CD133 and CD166[44,45]. In some cancers it has been observed that *Hox* genes are upregulated which are normally restricted to undifferentiated or proliferative cells, and *vice versa* a down-regulation has been shown of those *Hox* genes which are normally present in fully differentiated cells[48]. For instance, *HOXA9* seems to play a role in acute myelogenous leukemia[49]. *HOXB7, HOXC6* and *C9* transcription factors function in intima formation and vascularization in vascular-wall multipotent stem cells, which is in line with vascularization being important for cancer metastasis[50]. Further, colon carcinomas show an overexpression of *HOXA4* and *HOXD10* which distinguishes colonic stem cells from colon carcinoma cells[51].

Induced pluripotent stem cells

Induced pluripotent stem cells (iPS) have similar characteristics as ESCs including pluripotency and immortality[52]. As can be seen in Table 1, both stem cell types are generally negative for *Hox* gene expression. iPS cells are generated by introducing four defined transcription factors into somatic cells, which are OCT3/4, SOX2, KLF4, c-Myc or NANOG, and Lim2b replacing the later two[53,54]. In the meantime, several somatic cells can be reprogrammed such as fibroblasts, hepatocytes, keratinocytes and circulating T-cells, also with a reduced set of transcription factors, ranging from four factors to one factor with respect of the target cell[55]. Both, ESCs and iPS cells are characterized by the pluripotency markers NANOG, OCT3/4, ERAS, ESG1 and ECAT as well as TRA-1-60[53,56]. As iPS cells can differentiate into the same lineages as ESCs, which is the differentiation into all cell types of the three germ layers[57], the *Hox* gene expression is similar as well. It is repressed as they are quiescent and up-regulated when the differentiation processes start[58].

HOX TRANSCRIPTION FACTORS IN VARIOUS DIFFERENTIATIONS

Hox expression in adipogenic differentiation

The understanding of adipose tissue as an organ with a detrimental effect on the whole organism, due to its secreted adipokines, led to increased studies during the last decades, since the number of obesity patients rises all over the world. A dysfunction of adipocytes in obese patients can, *e.g.*, lead to diabetes type Ⅱ and cardiovascular diseases[59], the leading cause of death in industrialized countries. Thus understanding the

molecular mechanisms in adipogenesis has therefore gained much importance.

Hox gene expression in developing adipocytes has been studied in the pre-adipogenic committed murine cell line 3T3-L1, where *Hoxa4, a7*, and *Hoxd4* are involved in the differentiation towards adipocytes[60]. In human adipose tissue-derived stem cells (hAT-SC), *HOXC8* expression is drastically down-regulated during adipogenesis and almost vanishes in mature adipocytes. However, the down-regulation does not arise from a change of RNA expression, but is rather due to post-translational modifications by miRNAs. Forced expression of *HOXC8* in hAT-SC inhibits adipogenesis to a certain extent, indicating that this *Hox* gene has a functional role during the differentiation of these stem cells towards adipocytes[61].

There is also evidence that *HOXA1, A4* and *HOXC4* are directly involved in the differentiation and further generation of human white and brown adipose tissue[62]. Additionally, the *Hox* gene expression pattern is varying in adipocytes derived from different fat deposits within the human body. This pattern is also reflected in the *in vitro* differentiation of human primary pre-adipocytes isolated from the respective region. For instance pre-adipocytes isolated from the abdominal fat depot show an increased expression of *HOXA5* and *HOXB8* during differentiation towards adipocytes, as compared to gluteal pre-adipocytes, where *HOXA10* expression is higher, and *HOXC13* expression is exclusively restricted to gluteal pre-adipocytes[63]. Due to this variation in the expression pattern, a general statement about the *Hox* gene expression in differentiating adipocytes would not be sensible.

Understanding the principles of Hox signaling in white and brown adipose tissue formation might help prevent health damage in obese patients, such as the development of diabetes and consecutive the metabolic syndrome.

Hox expression in chondro-osteogenesis

Understanding the development of joints and bones is of interest in many fields of research, including regenerative medicine. However, the complete formation of limbs and other hard tissues involves various types of cells and a complex interplay between them. Three main tissue types can be attributed to bone and joint formation, namely tendons, cartilage and bone. Chondral tissue is the first to develop in limb formation, and its condensation is needed for correct bone development. Of great importance in tissue engineering are the underlying signaling pathways and proteins involved[64]. By investigating the *Hox* gene expression in chondrocytes and osteoblasts, both, *in vitro* and *in vivo*, bone formation and regeneration might be improved in the future, since in both cell types *Hoxa2* and *Hoxd9* have been shown to be significantly regulated. *Hoxa2* is up-regulated in bone regeneration of rats after post-fracture day 10, whereas *Hoxd9* is down-regulated after this time period[65]. In mice, *Hoxa2* impairs chondrogenesis in the mesenchymal-to-chondrocyte differentiation step leading to a proportionate short stature. Additionally, in a Hoxa2 gain-of-function mice model molecular regulators for an endochondral ossification are decreased[66]. In normal mouse development, Hoxa2 inhibits chondrogenesis and intramembranous ossification as well as bone formation when forcedly expressed in osteoprecursors[67]. Forced *Hoxa2* expression in cells committed to undergo chondrogenesis also leads to chondrodysplasia and delayed cartilage mineralization and ossification, but does not affect proliferation of the chondrocytes[68]. Interestingly, Cbfa1 induction might inhibit *Hoxa2* caused impairment of osteogenic differentiation *in vivo*[67]. In addition, overexpression of *HOXA2*, and expression of *HOXA3* and *HOXB4*, abolishes the ability of neural crest cells in the rostral domain to differentiate into skeletal structures in chicken[69]. The expression of certain *Hox* genes seems to inhibit skeletogenic differentiation, particularly jaw formation. In cranial neural crest cells there are specific regions where *Hox* expression or suppression, respectively is essential for appropriate development. In detail *Hoxa2* inhibits *Sox9*, resulting in an impeded development of neural crest cells into ectomesenchymal stem cells and thus no osteochondral progenitors can form in mice. The absence of *Hoxa2* leads to a migration of Sox9 into areas where *Hoxa2* is normally expressed which induces ectopic chondrogenesis[70]. In addition, *Hox* genes suppress osteochondrogenesis in more posterior neural crest cells *via* epigenetic mechanisms[32]. *Hoxa2* exerts an inhibitory effect in differentiation of chondrocytes, but is needed during bone formation. This effect is not related to the patterning function of *Hoxa2*, since it occurs in various tissues independent of their embryonic origin. Moreover, the ossification process itself is not affected by the *Hoxa2* misexpression[68].

Hoxc8 as well as *Hoxd4* regulate chondrogenesis in a dose dependent manner in mice. *Hoxc8* does not block chondrogenesis, but alters the progression of chondrocytes and their differentiation pathway, leading to an accumulation of proliferating precursor cells, which negatively interfere with the progression of differentiation. A transgenic model for *Hoxc8* and *Hoxd4* signaling in chondrogenesis has been suggested, but the proposed effects have not yet been proven in *in vitro* culture systems with postnatal derived chondrocytes[71,72].

In *Hoxa11* and *Hoxd11* double mutant mice, chondrogenesis is initiated normally. Pre-chondrocytes express Sox9 and type Ⅱ collagen necessary for further differentiation. However, mesenchymal condensations, which later give rise to the radius and ulna, differ in shape and size from non-mutants[73]. Prehypertrophic cells within these condensations do no longer undergo their differentiation pathway towards hypertrophic chondrocytes. *Hoxa11* and *Hoxd11* double deficient

mice have shorter limb formation and development postnatal, together with delayed ossification, suggesting that these two *Hox* genes are important regulators in mammalian chondrogenesis[73]. This is in line with the finding that *HOXA11* is expressed only in tissues which do not undergo chondrogenesis in chicken[74].

Also, *Hox* genes generate a positional memory in skeletal stem cells, influencing their differentiation potential. Murine skeletal progenitors derived from the mesoderm maintain *Hoxa11* expression, whereas mandibular bone progenitors derived from the neural crest are negative for *Hox* expression. Thus an appropriate expression of *Hox* genes provides instructions for site-specific differentiation and function of stem cells *in vivo* and *in vitro*[75].

Similar as already discussed for the adipogenic lineage commitment, the expression pattern of *Hox* genes in stem cell differentiations towards the osteogenic lineage varies according to the extraction site of the adipose tissue-derived stem cells. *Hoxa4* and *a5* expression is decreased in flank-derived MSCs differentiated towards osteoblasts, compared to the level in abdomen-derived stem cells, but *Hoxa4* is dominantly expressed in arm-derived stem cells. *Hoxc8* and *Hoxd4* on the other hand show a similar expression pattern in stem cells derived from the abdomen, arm, flank or thigh[76].

Genes within the D family of the *Hox* cluster are suggested to play a role in the condensation of MSCs and the further development into chondrocytes. In detail, *HOXD9* and *HOXD13* were found to be distinctly expressed in developing chondrocytes within MSCs from the chicken limb bud. The inhibition of *HOXD10, D11*, and *D13*, respectively, inhibits chondrogenesis[77]. This is in line with the finding that *HOXD11* was found to act on initial cartilage condensation phases and later growth phases within the bone of chicken, whereas *HOXD13* acts at later stages of bone development. Correspondingly, misexpression of *HOXD13* is associated with a shortening of long bones[78]. This is supported by data derived from mice where Hoxd13 and to a lesser extent Hoxd12 were found to be essential for bone formation. A defect in one of these Hox genes leads to limb malformations and shorter bones[79]. Especially *Hoxd13* seems to be responsible for the genetic switch from long bone to short bone formation[80]. But also *Hoxd10* affects mouse limb development by impairing proper growth of skeletal elements[81]. Large fractures heal by processes very similar to bone formation during development, apart from remodeling and inflammatory responses[5].

The strict *Hox* gene pattering that could be observed in Drosophila melanogaster is even more complex during bone formation in vertebrae. In *D. melanogaster* genes closer to the DNA 5′-end are expressed strictly posterior and the more 3′ located *Hox* genes are strictly anterior. In agreement with that a knockout of *Hoxd9* has revealed a more posterior impaired growth in the spine of mice than a knockout of Hoxd13[82].

As already mentioned *Hoxd13* is more essential for proper bone formation than *Hoxd12*, but defects in either of these *Hox* genes lead to a shortening of bones and limb malformations[79,81]. However, functions missing in knockout mice lacking a certain *Hox* gene may be compensated by other *Hox* genes. Hence, the severity when lacking either one of two *Hox* genes may be far less than if both *Hox* genes are knocked-out[82,83]. Cases where a *Hox* gene on both alleles is dysfunctional always show more severe bone malformations[79,81]. Also, stem cells from various sources show an origin-specific Hox expression profile. Therefore, it is of great importance to match the *Hox* code of the cells subject to transplantation to the tissue of interest. It has been shown that USSCs, which are *in vivo* Hox negative and lack the potential to differentiate into the adipogenic lineage, gain this potency after these cells have been altered in their *Hox* expression profile in a co-culture model with CB-MSCs or bone marrow-derived stem cells. This however also affects the chondrogenic and osteogenic differentiation potential of USSCs[84]. The positional memory may hence be altered under the influence of *Hox* genes, but side effects have to be taken into consideration.

An indirect link between *Hox* expression and bone formation was examined recently. BMP4 (bone morphogenic protein) as well as BMP7 elevates the expression of KDM6B, which then causes a removal of H3K27me3 that normally inhibits *Hox* expression. Even though BMP is among other tasks directly responsible for bone formation this secondary function might further aid in the development[85].

A well understood example for how *Hox* genes are involved in bone formation is *HOXA10*. Again a BMP, here BMP2 induces *HOXA10* which subsequently interacts directly with RUNX2, a main and early regulator of osteogenesis-specific gene expression[86,87]. This is supported by the finding of Gordon and colleagues[88] that Pbx1 is able to repress this *Hoxa10*-mediated osteogenic differentiation by blocking recruitment of chromatin remodeling factors and that overexpression of *Hoxa10* increased the expression of osteoblast-related genes, osteoblast differentiation and mineralization[89].

Once bone and cartilage have been formed, they are connected to the musculature *via* tendons. Influencing the proper development and regeneration of tendons and striated muscle is therefore of great medical importance. However, little is known to date about the involvement of *Hox* genes in their developmental processes. The first and only data are about the autopodal *Hox* genes *HOXA13* and *HOXD13* inducing Six2 expression, which is a transcription factor present in developing tendon precursors, even in areas where it normally would not occur, such as the zeugopods of a chicken limb-bud[90]. Further investigations are therefore required to be able to improve overall limb regeneration with the help of stem cells under the influence of *Hox* genes.

Hox expression in cardiovascular lineages

Regeneration and repair of the cardiovasculature is a major field of research in the scientific community, since it is the main cause of death with up to 50% in industrialized countries[91]. After transplantation of human bone marrow-derived stem cells into a murine adult heart, a limited number of cells survived and over time showed morphological and molecular changes, resembling the phenotype of host cardiomyocytes[92]. This has been reproduced in various studies and with a variety of donor tissues and hosts *in vivo* and *in vitro*[93,94]. Thus MSCs hence provide a valuable source for repairing injured myocardium in theory, but the underlying mechanism of differentiations towards myocytes and the cells of the blood vessels, *e.g.*, endothelial and smooth muscle cells, has to be understood thoroughly to improve the outcome of such treatments. Knowing about changes in *Hox* gene expression in stem cell types and animal models may in the future help improve the differentiation of MSCs into cardiomyocytes for the transplantation into patients.

HOXA1 deficiency can cause cardiovascular diseases in mouse models as well as in human patients, which suggests its role in cardiomyocte differentiations[75,94-98]. TCDD can inhibit mouse embryonic stem cell differentiation towards cardiomyocytes. During this inhibition *Hoxa10, a13, a7, Hoxb9, Hoxc8, c9, Hoxd1, d4, d9, d13* are upregulated, whereas *Hoxb1* is down-regulated. In detail, *Hoxb4* and *Hoxd3* are upregulated with a lower dosage of 10 pmol/L TCDD and down-regulated at a dosage of 100 pmol/L TCDD. *Hoxa9, Hoxb3, b8, Hoxc6* are down-regulated at a dosage of 10 pmol/L TCDD whereas upregulated at a dosage of 100 pmol/L TCDD[99]. In accordance to these findings, *Hoxa10* has been found to impair murine cardiac differentiation *in vivo*[100].

In the vasculature, *HOXA11* and *A13* impact myogenic differentiation, as they repress MyoD expression and therefore initiation of myogenic differentiation in chicken limb muscle precursors transfected with these *Hox* genes[101]. Retention of *Hox* gene expression profiles and positional identity has been found in muscle cells and MSCs *ex vivo*[75,95-98].

The genes of the paralog groups *Hox6-10* show a higher expression pattern in smooth muscle cells (SMCs) of the athero-resistant thoracic aorta compared to SMCs of the athero-susceptible aortic arch in mice, rat, and pork. This pattern is consistent in human ESCs which were differentiated into precursors of the thoracic aorta and the aortic arch, respectively, which also suggests a possible involvement of the above mentioned *Hox* genes in specific SMC differentiation processes of ESCs[102]. In vascular wall-resident multipotent stem cells (VW-MPSCs), *HOXB7, HOXC6* and *C8* are expressed in high levels compared to human pluripotent ESCs, mature aortic smooth muscle cells and human umbilical cord endothelial cells[50]. Silencing of these *Hox* genes in VW-MPSCs leads to a reduction in their in-gel (Matrigel) sprouting capacity, hence suggesting a decreased differentiation potential without affecting proliferation[50]. Finally it has been found that in myogenic cells *Hox* gene expression is down-regulated *via* hypermethylation[103].

Changes in *Hox* gene expression over the differentiation time course can also be observed in the stem cell differentiation towards endothelial cells. When mouse ESCs are differentiated towards endothelial cells, *Hoxa3* and *Hoxd3*, which drive angiogenesis and endothelial cell sprouting in adults, show a peak expression at day three of differentiation. Their expression declines over the following days of differentiation, whereas *Hoxa5* and *Hoxd10* arise from a low expression profile at day three of differentiation to a high expression profile over the course of time. In addition, these two *Hox* genes are involved in maintaining a mature quiescent endothelial cell phenotype[22]. This suggests an involvement of *Hoxa5* and *Hoxd10* in the differentiation of ESCs towards endothelial cells, whereas *Hoxa3* and *Hoxd3* rather mark an immature angiogenic endothelium and/or the onset of angiogenesis[22]. This has been confirmed by others who found that *Hoxd10* suppresses angiogenesis, while *Hoxd3* and *Hoxb3* promote angiogenesis[104,105].

Interestingly, the expression profile of *Hox* genes in human ESCs from leukemia prone infants is changed which might account for the impairment of hematopoietic cell differentiation in favor of endothelial cell fate. In these cells, *HOXA9, A13, HOXB2, B3, B4, B5,* and *B6* are strongly up-regulated[106]. Next to this, *HOXA9* is down-regulated in endothelial cells derived from endothelial progenitor cells from systemic sclerosis patients, suggesting an involvement of this *Hox* gene in endothelial cell differentiation or mal-differentiation[107].

During the differentiation of human bone marrow-derived MSCs towards endothelial cells four *Hox* genes were found to be significantly altered in their expression profile. *HOXA7* and *HOXB3* expression is increased, whereas the expression of *HOXA3* and *HOXB13* ceased[108]. Also, *HOXB5* up-regulates vascular endothelial growth factor receptor-2 which leads to an augmented endothelial cell precursor differentiation. Consequently, *HOXB5* has also been proven to enhance endothelial cell sprouting and coordinated vascular growth in chicken[109]. A variety of *Hox* genes have already been found to influence call fate in favor of endothelial and smooth muscle cell lineages, but the exact mechanisms need to be made clearer in order to use them as transcription factors in regenerative therapies.

Hox expression in neurogenesis

Neurodegenerative diseases are also becoming more and more prominent within an aging population. Repairing the neurological systems within the body has been difficult ever since, due to its complexity. A detailed understanding of molecular processes involved in the development and differentiation of neuron precursors is of crucial

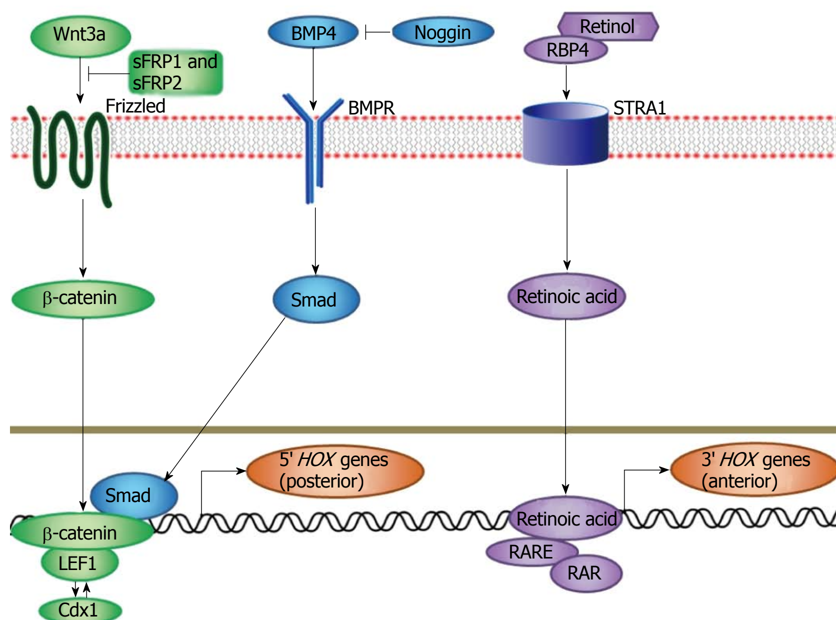

Figure 2 Major signaling pathways involved in *Hox* gene expression. Predominantly posterior 5' *Hox* genes are activated by BMP and Wnt signaling. *Hox* genes closer to the 3' end of a chromosome, which are mainly expressed anteriorly, are activated by retinoic acid pathways[120,141-150]. BMP: Bone morphogenic protein; RARE: Retinoic acid response element.

Table 2 *Hox* genes play a role in various differentiations

Differentiation	*Hox* gene expression	Ref.
Adipogenesis	*a4, a7, d4, A1, A4, A5, A10, B8, C4, C8* ↓, *C13*	[60-63]
Chondrogenesis	*a2* ↓, *a11, c8* ↓, *d4* ↓, *d11, D9* ↑, *D11, D13* ↑	[65,66,70-73,77,78]
Osteogenesis	*a2* ↑, *a10, d9* ↓, *d12, d13, A10*	[82,86,87,89]
Tendon differentiation	*A13, D13*	[90]
Cardiomyocyte differentiation	*a10, A1*	[75,95-98,100]
Smooth muscle cell differentiation	*a6-10, b6-10, c6-10, d6-10, A11, A13, B7, C6, C8*	[50,101,102]
Endothelial cell differentiation	*a3* ↓, *a5* ↑, *d3* ↓, *d10* ↑, *A7* ↑, *A9* ↑, *A13* ↑, *B2* ↑, *B3* ↑, *B4* ↑, *B5* ↑, *B6* ↑, *B13* ↑	[20,106-108]
Neurogenesis	*a2, b1, B4*	[110-112,114-116]

Small capitals: Murine; Large capitals: Human and chicken; ↑: Up-regulation; ↓: Down-regulation.

importance. Since *Hox* genes control the anterior-posterior (AP) segmentation, the 3' genes in the *Hox* cluster are more prone to play a role in neurogenesis, and the paralog groups 1-4 have restricted expression borders in the hindbrain. The remaining paralog groups 5-13 reveal an anterior expression border in the spinal cord. This AP segmentation translates to the migration of neural crest cells[12].

In mouse models, *Hoxb1* is essential for the correct development of rhombomeres at early embryonic stages, since it naturally induces neural stem cells towards a hindbrain identity, and has also been shown to be involved in the differentiation, maturation and maintenance of facial nerves[110,111]. Murine *Hoxa2* and *Hoxb1* can individually and as the sole additional factor induce motor neuron development, even in sections where they are usually not present[112]. In mice deficient for *Hoxb1* expression, facial neurons do not properly form[113]. Also, *HOXB4* has been found to be a crucial factor in driving neuronal differentiations in the neural tube[114]. Further, since the disruption of *Hoxa1* in mice leads to malformations of the hindbrain, a role for this *Hox* gene in murine neurogenesis has been suggested[115,116]. Retinoic acid (RA) has been established as an essential factor for adult neurogenesis. Embryonic stem cells treated with RA almost exclusively differentiate into neurons and develop a Hox expression profile[117]. Many *Hox* genes contain retinoic acid response elements (RAREs) which supports the finding that they are involved in RA-induced differentiation of ESCs[118]. RAREs have been found in *Hoxa1, a4, Hoxb1, b4*, and *Hoxd4*[12,119-128]. In fact, several *Hox* genes have been found to be strongly up-regulated in differentiations where RA was present[118]. The interaction between RA, *Hox* genes, and other signaling molecules has been visualized in Figure 2. During embryonic stem cell differentiation, highly conserved elements in the *Hox* gene clusters are epigenetically modified, so that adult spinal cord progenitors express a distinct *Hox* expression profile[129-131]. The further investigation of *Hox* genes in neurogenesis therefore promises a better insight into the possible

therapies to treat neurodegenerative diseases and should be pursued.

CONCLUSION

Degenerative diseases are one of the major problems arising within a population with high average age. The complex biological systems in the human body have made the development of regenerative therapies a challenging project. Much effort has been put into using stem cells and various signaling molecules for this purpose over the last decades. In this review, we have discussed the expression of *Hox* transcription factors in various types of stem cells and their role in differentiation. Interestingly already the stem cell source has an impact on the *Hox* gene expression in the respective stem cell type. *Hox* genes provide valuable possibilities to influence stem cell differentiations. Either single *Hox* genes or small groups have been found to directly influence the course of several differentiations, but detailed molecular changes under this influence are mostly not yet known. Additionally, many *Hox* genes have only revealed an indirect effect, or have been investigated in *in vivo* development but not in stem cell differentiations. Future studies need to be performed to investigate whether the suggested *Hox* genes, as summarized in Table 2 for mesenchymal stem cell lineages, actually have a functional role in the *in vitro* differentiation of stem cells. Additionally, the effects must be tested *in vivo* to evaluate their therapeutic potential in tissue repair and regeneration[132]. As a consequence of the observed results it is suggested that cells which have their *HOX* code deleted regain plasticity and can be reprogrammed, similar as it has already been shown in iPS cells[133,134]. In addition to this and similar to iPS cells, also the epigenetic profile must be considered. We summarized that next to the histological compatibility of donor and host tissues the *HOX* code must be taken into consideration in regenerative therapies. In conclusion, the further investigation of *Hox* genes in stem cell differentiations is an interesting research field in regenerative medicine to improve tissue repair in injury, disease and aging.

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