Chapter

Organ- and Site-Specific HOX Gene Expression in Stromal Cells

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Abstract

HOX genes are a group of evolutionarily conserved genes that encode a family of transcription factors that regulate early developmental morphogenetic processes and continue to be expressed into adulthood. These highly conserved HOX factors play an unquestioned crucial role as master regulators during embryonic vertebrate development and morphogenesis by controlling the three dimensional body plan organization. HOX genes specify regions of the body plan of an embryo along the head-tail axis. They encode proteins that specify the characteristics of 'position', ensuring that the correct structures form in the correct places of the body. Expression of HOX is known to persist in many tissues in the postnatal period suggesting the role of these genes not only during development but also for the functioning of tissues throughout life. The tissue-specific pattern of HOX gene expression is inherent in stromal/stem cells of mesenchymal origin, such as mesenchymal stromal cells, fibroblasts, smooth muscle cells, and preadipocytes, enabling them to memorize their topographic location in the form of their HOX code and to fulfill their location-specific functions. In this chapter, we focus on the expression and potential role of HOX genes in adult tissues. We review evidence that site-specific expression of HOX genes is connected to location-specific disease susceptibility and review studies showing that dysregulated expression of HOX genes can be associated with various diseases. By recognizing the importance of site-specific molecular mechanisms in the organ stroma, we gain new insights into the processes underlying the site-specific manifestation of disease.

Keywords: Fibroblasts, HOX genes, site-specific gene expression, embryonic development, disease locations

1. Introduction

In vertebrate animals, the stromal compartment of organs is composed of extracellular matrix and mesenchymal cells. Fibroblasts are one of the most abundant and principal stromal cell types and have a variety of vital, locally specialized functions in tissue repair and homeostasis. Primarily, they are the main source of extracellular matrix (ECM) proteins, which, in addition to providing a structural scaffold for cells, play critical roles in determining cell phenotype and function. Fibroblasts produce and secrete all components of the ECM, including structural proteins, adhesive proteins, and a space-filling ground substance composed of glycosaminoglycans and proteoglycans [1].

Furthermore, as a result of their reciprocal interaction with epithelial cells, fibroblast cells play an important role during development and morphogenesis of tissues and organs including the skin, eyes, lung and other visceral organs. The organ stroma provides a structural framework and guidance for blood and lymphatic vessels, nerves and leukocytes and is critically involved in the regulation of physiological organ function.

During stress, fibroblasts respond by sending signals to help the surrounding tissue to adapt to changes in the environment [2]. The phenotype of fibroblast can transform and provides the necessary components to replace wounded tissue. During pathologic states, however, ECM can be generated in excessive quantities, leading to irreversible organ dysfunction caused by collagen deposition in a dysregulated manner [3].

Given the wide variations of gene expression and strikingly different responses to extracellular signals among fibroblasts from different organs, these fibroblast populations should be considered as distinct cell types. Human fibroblasts obtained from vocal fold, trachea, lung, abdomen, scalp, upper gingiva, and soft palate, displayed a phenomenon of global topographic differentiation across all anatomic site domains with the specialized genotype of the vocal fold fibroblast uniquely characterized to achieve homeostasis under complex mechanobiological requirements [4].

During development of multicellular organisms, developmental control genes are critical for pattern formation and cell fate specification in specific spatiotemporal patterns [5]. Most of these genes encode transcription factors acting in cascades and networks, regulating the expression of further developmental control genes and ultimately organ-specific 'effector' genes, which control patterning, morphogenesis and differentiation of tissue-specific functions and specific body parts [6]. The wide range of organ specific differences in fibroblast function is complemented by distinct and characteristic gene expression patterns depending on their anatomic site of origin [7]. Examples include HOX genes, which can lead to the transformation of specific body segments when mutated [8], Pax6, which controls eye development [9] and MyoD, which is crucial for muscle formation [10].

Functional diversity of fibroblasts is not only important during embryonic developmental and physiologic specialization of many tissues, but might also influence site- and organ-specific differences in the susceptibility of different tissues to disease development [11, 12].

2. Site-specific regulation of gene expression by HOX genes

Spatial organization of cellular differentiation is achieved by a unique local developmental specification of cell types. This can be reinforced by the cells' interpretation of environmental signals specific to their position in the body. HOX genes are known to be master regulators of body pattern formation during embryogenesis, activating other genes to specify positional identities in development.

In humans, there are 39 HOX genes organized into 4 distinct clusters. The 4 clusters map to 4 different chromosomes and contain between 9 and 11 genes. These clusters, labeled HOXA, HOXB, HOXC and HOXD, are located on chromosomes 7p14, 17q21, 12q13 and 2q31, respectively [13, 14]. HOX proteins are transcription factors that are bound by different protein cofactors. The analysis of HOX protein binding activity showed that the homeodomain, a highly conserved 60 amino acid helix-turn-helix motif, is a DNA-binding protein that recognize AT rich short DNA motifs, often

with a core of TAAT [15]. In several cases, functional specificity of the different HOX proteins could be attributed to the homeodomain itself [16, 17]. The homeodomains showed distinct protein- and/or DNA-binding activities, suggesting that variation in sequence recognition may be a factor in their functional diversity.

In vertebrates, the different sets of HOX genes are expressed in parallel [15]. Thus, site- and/or organ-specific fibroblast phenotypes are shaped by a combinatorial expression of genes from the four HOX clusters. Genome-wide gene expression profile of 47 fibroblast populations from 43 anatomic sites spanning the human body, including arm, leg, trunk, foreskin and internal organs, analyzed by unsupervised hierarchical clustering revealed a specific code of HOX genes according to their position in the human body. Fibroblasts from the same topographic site, independent from which organ they were isolated, expressed the same, site-specific, unique "HOX-code", which resulted in grouping of the cells from the same site together, based solely on the expression pattern of the HOX genes. This suggests that the expression of a specific set of HOX genes is connected to the cells' location in the body, potentially conferring important site-specific functions needed in a particular location [18].

Thus, site-specific variations in the gene expression programs of fibroblasts are not random but are systematically related to their positional identities along the major anatomical axes, which are formed during embryogenesis.

3. The role of HOX genes in embryonic development

In many animals, especially vertebrates, various HOX gene paralogues are located genetically close to each other in clusters [19]. The order of the genes on the chromosome corresponds to the expression of the genes in the developing embryo [14, 20, 21]. Thereby, the genes in the 5′ genetic locus are expressed in the anterior end or distal parts of the developing organism and the genes in the 3′ locus are expressed in the posterior end or proximal parts - a phenomenon known as spatial colinearity [22]. In addition, there is a temporal sequence of activation of HOX genes in vertebrate. 5′ genes are expressed later, whereas 3′ genes are expressed earlier in embryonic development [18, 19]. The unique orderly arrangement of the genes in the genome is related to the activation of HOX genes in a temporal sequence by gradual unpacking of chromatin along a gene cluster, thereby controlling the expression of the right HOX gene at the right location at the right time in embryogenesis [23]. The temporal and spatial tightly controlled expression of HOX genes in mesenchymal cells can thus explain how genetic information is translated to the spatial organization of various cells in the body (**Figure 1**).

Over the past years, gain-of-function and loss-of-function approaches in chick and mice together with studies of mutations, indicated the essential role of HOX genes in proper vertebrate limb growth and organization of the structures within both the anterior-to-posterior axis and the proximal-to-distal axis. The resultant phenotypes typically follow the expected spatio-temporal expression of HOX genes with more 3′ genes causing malformations in more anterior and proximal areas and more 5′ genes causing defects in more posterior, distal body segments and organs. For instance, Hoxa13 and Hoxd13 have been identified to be involved in endochondral bone formation [24]. HOXD13 is mutated in synpolydactyly, and HOXA13 is mutated in Hand-Foot-Genital syndrome [22]. Overexpression of Hoxa13 affects the expression of Enpp2, an enzyme produced in precartilaginous condensations that modulates cell motility [25]. Loss-of-function of Hoxa13 was also reported to modulate expression of

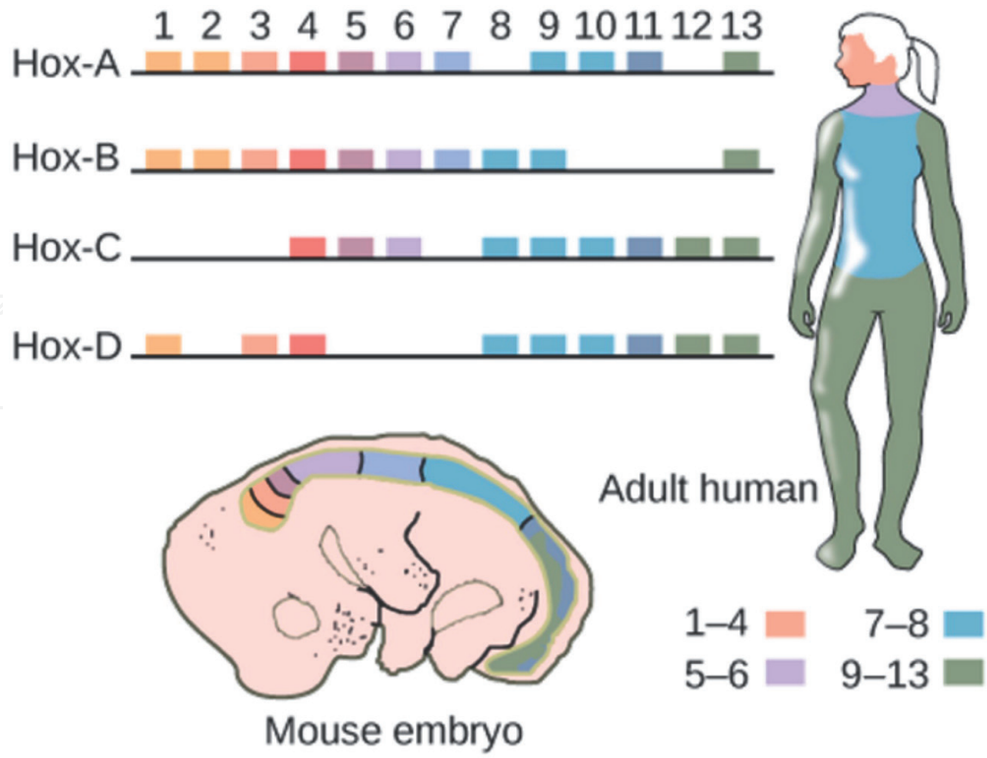

Figure 1.
The spatial organization of the Hox genes in the various genomic Hox clusters (Hox-A, Hox-B, Hox-C and Hox-D) is tightly linked to the pattern of their expression in the embryo (mouse embryo shown here). The same pattern of HOX gene expression that formed during embryonic development can still be found in stromal tissues (fibroblasts, endothelial cells) in the adult human body. The picture was taken from the course 'Homeotic genes' by the Khan Academy. Note: All Khan Academy content is available for free at www.khanacademy.org.

bone morphogenetic proteins like BMP2 and BMP7 [26]. Hoxa13 regulated expression of BMP2 and BMP7 was shown to control distal limb morphogenesis [27].

Temporal and spatial dynamic regulation of gene expression is a hallmark of developmental control genes, since they have to act locally in order to affect specific developmental processes [28]. By linking differentiation programs to specific cell positions, selected cells can be programmed to develop similarly in a defined region. While spatial boundaries are first defined during embryonic development, these spatial patterns of cellular specialization also need to be maintained throughout adulthood as the tissues undergo continuous self-renewal.

4. Epigenetic regulation of HOX gene expression

Epigenetic mechanisms have an important role in the regulation of HOX gene expression in embryonic development and in differentiated cells. The established expression patterns of HOX genes must be precisely and clonally maintained throughout development. Epigenetic regulation of gene expression by chromatin structure is defined by a set of posttranslational modifications of histones, such as methylation, acetylation and phosphorylation. Furthermore, gene expression can be epigenetically regulated by DNA methylation.

In totipotent embryonic stem cells, HOX genes are silenced and then rapidly activated during embryogenesis. In drosophila and vertebrates, repressive Polycomb and

Trithorax group complexes regulating histone methylation control the proper maintenance of HOX gene expression during this process [29]. Tight temporal and spatial control of HOX gene expression in this phase is essential. In vertebrates, dynamic changes in histone methylation are observed during the sequential activation of HOX genes in the embryo, suggesting that progressive change of epigenetic modifications regulate collinear gene activation in these loci [29]. Based on the presence of retinoic acid response elements (RAREs) in the regulatory region of many of the HOX genes, it was shown that that retinoic acid is a crucial factor in the epigenetic regulation of histone methylation of the clustered HOX genes [30].

During developmental stages, the genetic loci of the 4 HOX clusters carry so-called "bivalent chromatin tags", like many other key developmental genes in embryonic stem cells [31]. This means that the HOX clusters possess at the same time "active" (i.e., trimethylation of histone 3 at lysine 4 -H3K4me3) and "inactive" (i.e., trimethylation of histone 3 at lysine 27 -H3K27me3) histone marks. In the presence of the right trans-acting factors, these bivalent tags can rapidly change to allow binding to cis-elements and initiation of transcription [32].

Epigenetic modifications also regulate tissue specific expression of HOX genes and mesenchymal cell differentiation [33]. In a study of genome-wide differential DNA methylation by reduced representation bisulfite sequencing (RRBS), the HOX gene clusters were highly overrepresented among the genes with hypermethylation in the skeletal muscle lineage [34]. Analysis of DNA methylation of HOX genes in myoblasts, myotubes and adult skeletal muscle tissue revealed that myogenic DNA hypermethylation of promoters and enhancers of HOX genes helps to fine-tune HOX gene expression in cellular differentiation [34].

HOX loci contain many noncoding transcripts. Long non-coding RNAs (lncRNAs) represent a class of noncoding RNAs that are longer than 200 nucleotides without protein-coding potential. They have been found to function as master regulators of gene expression in health and in various human diseases. LncRNAs can regulate biological functions in cis and in trans [35]. For instance, they can recruit histone modifying enzymes to specific genomic sites and serve as scaffolds for gene expression modulating enzymes [36].

HOX antisense intergenic RNA (HOTAIR) is a lncRNA that is encoded in the HOXC locus and that mediates the placement of repressive H3K27me3 marks. HOTAIR expression has been shown to repress the expression of genes in the 3' HOXD locus and thus was suggested to be an important epigenetic regulator of site-specific HOX expression during embryonic development [37]. However, these data is debated and the full role of HOTAIR in embryonic development is not yet understood [38].

HOXA transcript at the distal tip (HOTTIP) is a 3764 nucleotide lncRNA encoded from a genomic region in the 5′ tip of the HOXA locus, regulating the expression of HOXA13 [39]. By binding the adaptor protein WD repeat-containing protein 5 (WDR5) and interaction with mixed lineage leukemia (MLL), HOTTIP can catalyze methylation of histone H3 lysine K4 (H3K4). H3K4 methylation is associated with increased gene transcription and MLL-WDR5 complexes were shown to occupy transcriptional start sites of various HOXA genes.

To date, the complex epigenetic patterns of cellular specialization in adult vertebrates and the mechanisms of their maintenance are not well understood. Epigenetic conservation of region-specific HOX gene expression in human adult fibroblasts suggests that they serve to maintain the differential patterns of the stroma during homeostasis and regeneration [40].

5. HOX gene expression in specific fibroblast populations

5.1 Skin fibroblasts

Epithelial tissues such as skin demonstrate remarkable anatomic differences leading to diversity on their structure and function. Genome-wide studies in skin fibroblasts demonstrated that site-specific HOX expression in these cells can define and maintain skin positional identity [41]. Analysis of genome-wide patterns of gene expression in cultured fetal and adult human skin fibroblasts at different anatomical sites showed distinct and characteristic site-specific transcriptional patterns, suggesting that fibroblasts at different locations in the body possess specialized functions [7]. The maintenance of region-specific HOX gene expression in adult fibroblasts may serve as a source of positional memory for skin during homeostasis and regeneration.

For instance, HOXA13, a gene that is preferentially expressed at distal body parts such as hands and feet, remains essential for maintaining the distal-specific transcriptional program in adult fibroblasts by inducing the expression of WNT5A, which is crucial for distal organ development [41]. Furthermore, reduction of HOXA13 abrogates the ability of distal fibroblasts to produce epidermal keratin 9, a distal-specific gene. Keratin 9 has been shown to be important for maintaining the mechanical integrity of palmar and plantar skin [42]. Together these observations suggest that HOXA13-regulated gene expression in adult human fibroblasts provide the specific functions of plantar and palmar skin.

5.2 Synovial fibroblasts

Synovial fibroblasts are crucially involved in inflammation and joint destruction in chronic joint inflammation (arthritis) [43]. Synovial fibroblasts isolated from different joint locations show substantial differences in their transcriptome and function, in particular a site-specific HOX gene signature was found [44]. Adult human and mouse SFs from different anatomical locations exhibit joint-specific HOXA and HOXC signatures that are maintained over several passages in cell culture conditions, are arthritis independent and reproduced in whole synovial tissues.

This HOX gene signature was shown to be epigenetically imprinted by DNA methylation and histone modifications [44, 45]. The joint-specific HOX expression in mouse and human synovial fibroblasts and synovial tissues reflected the pattern of HOX gene expression during embryonic limb development [44]. Only few studies explored the functional role of different HOX proteins in synovial fibroblasts. HOXD10 silencing downregulated the p38/c-Jun N-terminal kinase signaling pathway, and suppressed the migration of synovial fibroblasts [26]. HOXD9 was found to modulate proliferation of synovial fibroblasts [46]. During development of the distal limb mesenchyme, Hoxd13 is the most strongly expressed HoxD gene, with a progressive decline in expression levels of Hoxd12 to Hoxd9 [47]. Moreover, in tetrapods, coordinated expression of the 5′ located Hoxd genes is essential for the development of digits [48]. Thus, site-specific expression of HOXD genes in distal joints (hands and feet) [44] might influence the activation of pro-inflammatory pathways and the migratory and proliferative capacity of synovial fibroblasts. Notably, distal joints are the first and most severely affected joints in patients with rheumatoid arthritis (RA).

Other HOX genes may also play a critical role in the pathogenesis of RA. One study showed that basic fibroblast growth factor (bFGF) affects the expression and transcriptional regulation of HOXC4 and via this pathway promotes hyperplasia of the synovium in RA [49].

Several studies suggest epigenetic changes as determinants of a persistent activated phenotype of synovial fibroblasts in RA [43]. RA synovial fibroblasts display wide-spread changes in DNA methylation causing up-regulation of disease relevant genes such as growth factors, adhesion molecules and matrix-metalloproteinases (MMPs) [50–52]. The tight epigenetic regulation of site-specific HOX gene expression might thus be disturbed in RA synovial fibroblasts, leading to aberrant expression of HOX genes at sites where they are normally repressed. The expression of HOXD10 for instance was found to be higher in knee synovial fibroblasts from patients with rheumatoid arthritis (RA) compared to osteoarthritis (OA) [26].

The lncRNA HOTTIP, encoded in the HOXA cluster, was shown to play a crucial role in the persistent activation of myofibroblasts promoting chronic inflammation and collagen deposition [53]. Silencing of HOTTIP reduced inflammation in a mouse model of arthritis and modified synovial fibroblast function by DNA demethylation of the locus encoding SFRP1 (Secreted Frizzled Related Protein 1), a modulator of the Wnt signaling pathway [54].

5.3 Gastrointestinal fibroblasts

The adult gastrointestinal tract was shown to keep the position-specific expression pattern of HOX genes along the anteroposterior axis of embryonic development, recapitulating the expression pattern in the embryonic gastrointestinal tract [55]. HOX gene expression varied over 11 different measured gastrointestinal sites and clearly separated segments of the upper gastrointestinal tract from segments of the lower gastrointestinal tract. Accordingly, differences in HOX gene expression were found by comparing the gene expression profile of gastrointestinal fibroblasts isolated from the stomach, ileum and the colon. In particular, HOX paralogs with lower numbers (e.g. *HOXA2*, *HOXD3*) were preferentially expressed in the esophagus and stomach, while HOX paralogues with higher numbers (e.g. *HOXA10*, *HOXD10*) tended to be more expressed in the cecum and rectum. Using hierarchical clustering analysis, different subgroups of gastrointestinal fibroblasts were identified based on differences in transcriptional regulation, signaling ligands, and extracellular matrix remodeling [56].

Gastrointestinal fibroblasts play a pivotal role in gastrointestinal epithelial renewal by supporting epithelial cell differentiation, and they have been described to contribute to gastrointestinal inflammation and fibrosis [57, 58]. Furthermore, gastrointestinal fibroblasts are strongly involved in the initiation, progression and metastasis of gastrointestinal cancer [59]. For instance, increased expression of HOXA13, *HOXB13* and *HOXC13* was found in esophageal pathologies such as esophageal squamous cell cancer, Barrett's esophagus or esophageal adenocarcinoma [60, 61]. Aberrant expression these HOX paralogues, which are normally less expressed in upper gastrointestinal parts might contribute to the activation of pathogenic processes at this site. Together these observations suggest that HOX genes might play a role in steering position-specific processes during gut inflammation and cancer development [62]. Unfortunately, up to know functional studies are lacking that analyzed the impact of site-specific HOX expression on gut homeostasis and disease development.

6. HOX genes in hematopoietic cells

Apart from stromal cells, HOX genes are expressed in hematopoietic stem cells and progenitors in early development, with a pattern characteristic of the lineage

and stage of differentiation of the cell. Using gene targeting technology and gain-of-function and loss-of-function mutations, the function of HOX genes in hematopoiesis has been extensively investigated [63].

For example, HOXB3, HOXB4 and HOXA9 are highly expressed in uncommitted hematopoietic cells, whereas HOXB8 and HOXA10 are expressed in myeloid committed cells. The different HOX clusters also have specific patterns of lineage-restricted expression, whereby HOXA genes are expressed in myeloid cells, HOXB genes in erythroid cells and HOXC genes in lymphoid cells. Intriguingly, the HOXD genes are not expressed in hematopoiesis despite having similar regulatory regions to the other clusters [64–66].

These observations indicate that modulation of the expression of a particular HOX gene can alter cell phenotype and suggest a causal relationship for lineage-specific patterns of HOX gene expression [67].

7. HOX genes in disease

7.1 HOX genes in cardiovascular disease

Steadily increasing evidence supports the idea that gene expression diversities in the vascular system are a major contributing factor in determining region-specific cardiovascular disease susceptibility. The regionally distinct and topographic expression patterns of HOX transcription factors in embryonic development is remembered in vascular smooth muscle cells [68] as well as in endothelial cells [7, 18, 69]. The persistent topographic expression patterns in post-natal vascular tissues suggest that HOX genes play a critical role in maintaining vessel wall homeostasis in a region-specific manner [70]. Intriguingly, in adult mice, high-throughput mRNA profiling revealed that HOX paralogues 6-10 (Hox6-10) are higher expressed in the thoracic aorta, which is resistant to atherosclerotic lesions, than in the aortic arch, which is highly atherosusceptible [68]. In humans, the differential expression of HOXA9 gene contributed to phenotypic differences in smooth muscle cells from athero-resistant compared atherosusceptible regions, which might be connected to the site-specific development of atherosclerotic plaques. For example, region-specific reciprocal interactions of HOXA9 with the pro-inflammatory transcription factor NF-κB have been demonstrated. In general, genetic regulatory networks of cardiovascular diseases processes implicated genes of various functional categories such as ECM remodeling, transmembrane signaling, cell cycle control, and inflammatory response as potentially HOX-dependent.

Ectopic activation of HOXC10 and HOXC9 in atherosclerotic coronary arteries has been found to be associated with loss of DNA methylation within the HOXC11/HOXC9 genomic interval [34, 71]. These data define epigenetic mechanisms controlling HOX expression as critical in aberrant expression of HOX and HOX-target genes in cardiovascular disease [72].

7.2 HOX genes in solid cancers

In embryogenesis, a fine balance between cell proliferation and differentiation is essential for normal development of the fetus, but in cancer, the balance between the two processes is impaired [63, 73].

Studies suggest that the expression of HOX genes becomes dysregulated during development of various solid tumors, including colon, breast, prostate, lung,

glioblastomas, thyroid, bladder, ovarian, melanoma, and kidney cancers [74, 75]. In fact, the specific pattern of change in HOX gene expression is dependent on cancer type, tumor stage, and, in certain cases, on anatomic location [76].

HOX genes have been identified to be important regulators of cancer stem cells (CSCs) which are critical for initiation and progression of solid tumors [33, 74]. These genes act as transcriptional activators as well as transcriptional repressors in cancers [77]. Studies show that the expression of specific HOX genes in cancers tends to differ based on tissue type and tumor site. HOXA genes were often reported to have altered expression in breast and ovarian cancers, HOXB genes in colon cancers, HOXC genes in prostate and lung cancer and HOXD genes in colon and breast cancers. This pattern can be linked to the embryonic origin of tissues. For example, colon, prostate, and lung, originating from endodermal, showed relatively similar HOXA and HOXB family gene expression patterns compared to breast tumors arising from mammary tissue, which originates from the ectoderm [74].

The differential expression of HOX genes in various solid tumors provides an opportunity to advance our understanding of cancer development and to develop new therapeutic agents. Specific methylation profiles in HOX clusters or in HOX-associated histones are recognized as potential biomarkers in several cancers and can be exploited in cancer therapy. The use of epigenetic drugs affecting generalized or specific DNA methylation profiles is a promising approach in cancer therapy in the near future. However, since the generalized effect of epigenetic drugs may lead to secondary malignancies, the development of drugs targeting specific epigenetic alterations, including those related to HOX genes, could advance this therapeutic approach [78].

HOX genes are recognized as potential therapeutic targets in adrenocortical tumors. Understanding the pathway being regulated by the transcription factor HOXB9, which promotes adrenal tumor progression through an increase in the expression of cell cycle genes, including Ccne1, could help to development potential drug targets for adrenocortical carcinoma [79]. Moreover, HOX peptide inhibitor showed a promising effect on cell survival in mice and could be used as peptide-based cancer therapeutics [80].

Analyses of HOX gene expression in normal breast tissue and primary breast cancers [81] showed that several HOX genes are differentially expressed in breast cancer compared to normal breast tissues, with different breast cancer tissues and cell lines showing high variability in the pattern of HOX gene expression [81]. Thus, these studies support the idea that aberrant expression of HOX genes is involved in the development of breast cancer and in the malignant behavior of cancer cells, but shows that, at least in breast cancer, there is no uniform pattern of HOX gene alterations that lead to malignant growth of cells.

The stroma surrounding solid tumors is built from cancer-associated fibroblasts (CAFs) which are recognized to play a significant role in tumor growth. Interestingly, it could be shown that CAFs predominantly develop from local fibroblasts at the site of the tumor [82]. Therefore, site-specific differences in local fibroblasts surrounding the tumor might be crucially involved in tumor development and invasiveness. Furthermore, site-specific differences in fibroblasts might be connected to the site-specific distribution of cancer metastases in certain organs [83]. This would support the "seed and soil" hypothesis, which states that the distribution pattern of metastases is highly dependent on the microenvironment of the organ in which the metastases are located [84]. Unfortunately, an analysis of the expression and influence of HOX genes in CAFs from different tumor sites is missing up to now.

7.3 HOX genes in joint and bone disease

Mammalian HOX genes are critical for proper development of skeletal morphology during embryogenesis. The continuous function of HOX genes in the skeleton after the establishment of skeletal morphology has been determined using genetic tools in mouse models [85]. The generation of a conditional Hoxd11 allele that can be deleted at adult stages after normal development and growth of the skeleton was used to show that Hox genes in the adult skeleton regulate the differentiation of skeletal stem cells into bone cells [86]. These data convincingly showed that Hox gene function in the skeleton is not restricted to development and that Hox genes play a crucial, functional role in adult bone homeostasis.

Furthermore, functional importance of Hox genes in the regulation of chondrocyte differentiation has been demonstrated [85, 87]. Hox genes are involved in the regulation of the progression of cells along the chondrogenic differentiation pathway after the initial formation of the cartilage anlagen. However, overexpression of a Hoxc8 transgene caused cartilage defects whose severity depended on the dosage of the transgene [85]. The abnormal cartilage was characterized by an accumulation of proliferating chondrocytes and reduced maturation. These results suggest that Hoxc8 continues to regulate cartilage homeostasis after development, presumably by controlling the progression of cells along the chondrocyte differentiation pathway. Their capacity for regulation of cartilage differentiation suggests that HOX genes could also be involved in human chondrodysplasias or other cartilage disorders [85].

Arthritic joints are characterized by rearrangements and dysregulated gene expression of bone, cartilage and synovial tissues [88, 89]. The exact molecular and cellular events leading to the development of the different kinds of arthritis still remain elusive, but involvement of HOX gene regulation in the key tissues affected by rheumatic muscular-skeletal diseases indicate a potential link between arthritis development and HOX transcription factors. HOX genes were for instance associated with the onset and development of osteoarthritis (OA) (e.g. HOXA9 in hip OA) and as mentioned above with pathogenic important joint-specific functions of synovial fibroblasts in RA [45, 90, 91]. However, it has not been clarified up to know whether changes of expression of HOX genes in OA are specific for a specific joint region or a common feature of disease.

Like a number of human diseases, rheumatic diseases include characteristic pathologies in specific anatomical locations [21, 44]. For example, ankylosing spondylitis is a chronic inflammatory disease affecting the spinal vertebrae and sacroiliac joints, causing debilitating pain and loss of mobility [92]. Joints in the hands, are commonly involved in RA and OA [93]. Reactive arthritis is a rheumatic condition that causes inflammation particularly in knees [94]. Several mechanisms might be involved in this susceptibility of specific joints for developing specific forms of arthritis. In addition to and maybe in combination with local mechanic factors, site-specific gene expression of local cell types (bone, cartilage, synovium), potentially regulated by HOX genes, might be crucially involved in the development of specific arthritides in specific joint locations. Furthermore, disease-specific systemic triggers, such as specific auto-antibodies or cytokines might preferentially affect local cell types at a specific anatomic sites [95].

8. Conclusions

HOX genes, a family of homeodomain transcription factors, guide embryonic development by encoding positional information during axis formation,

determining site specificity of the body plan and regulating formation of structures along the various body axes.

Like other transcription factors, HOX genes control the establishment and maintenance of specific cell states by regulating distinct sets of downstream genes. Despite their similar DNA-binding properties, they have highly specific effects on the transcriptome. This genetic control results in functional diversity of various body parts. Cells such as fibroblasts thus develop not only an organ-specific but also a site-specific transcriptional program. Intriguingly, these HOX regulated transcriptional programs are epigenetically maintained in adult cells.

Several studies endorse that regulation of site-specific gene expression by HOX genes contribute to the development of a broad range of diseases (**Figure 2**). The site-specific environment created by the expression of specific HOX genes might promote or prevent the development of diseases at specific locations, in line with the 'seed and soil' hypothesis. Furthermore, activation or repression of HOX expression during disease development, potentially by modulation of the epigenetic mechanisms regulating the HOX loci, might further influence site-specific disease processes.

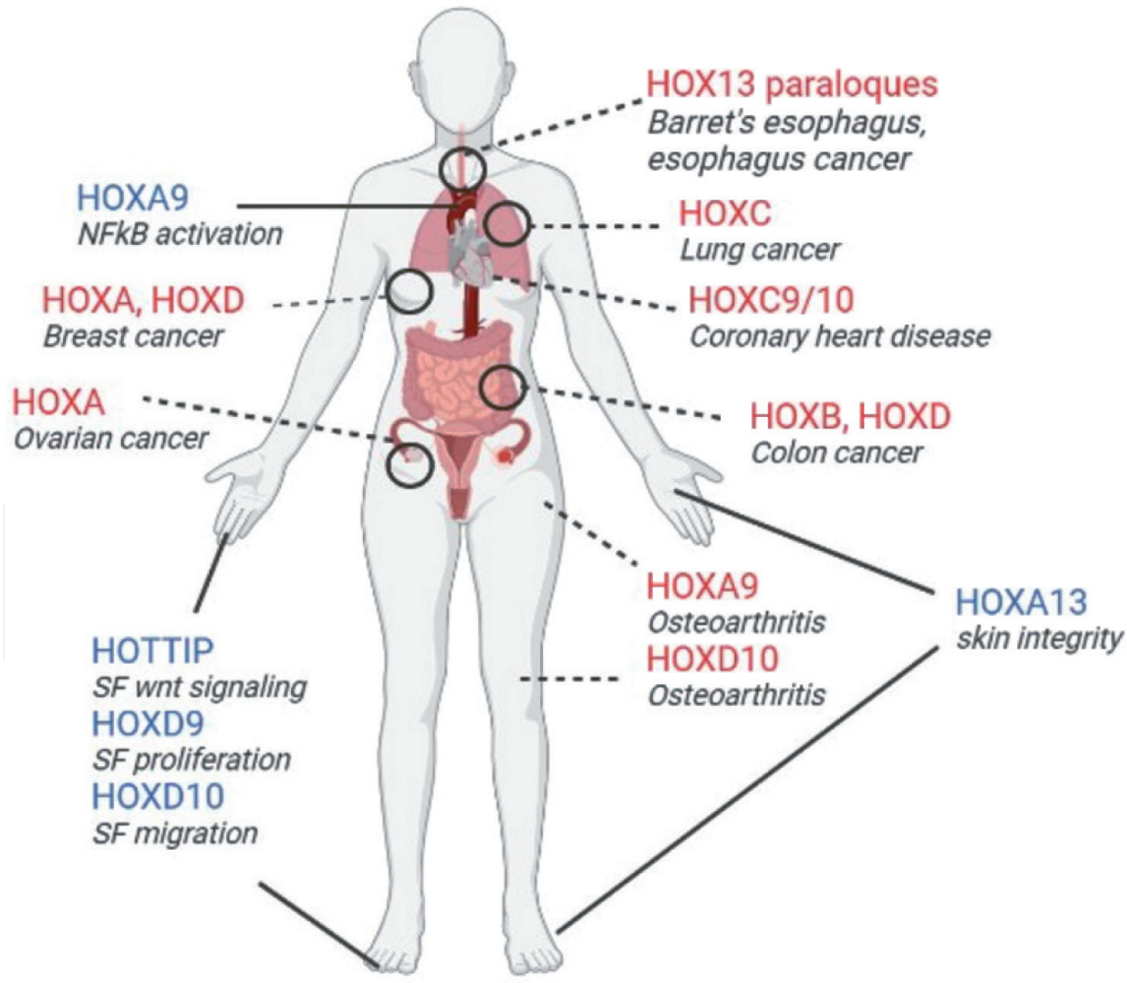

Figure 2.
Summary of diseases that have been associated with HOX gene expression. In some cases (blue letters) regular site-specific expression of HOX genes has been connected to disease development based on the functions of the respective HOX genes (below in italics). In other cases, aberrant expression of HOX genes (red letters) was associated with the development of diseases (below in italics). SF = synovial fibroblasts. The figure was created with Biorender.

Therefore, a better understanding of site-specific cellular and molecular mechanisms underlying regional appearance of disease is essential for understanding disease development and designing new therapeutic approaches.

Conflict of interest

The authors declare no conflict of interest.

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