Review Article

Human Homeobox Genes in Development and Cancer

Abstract

The homeobox (HOX) genes family encode highly conserved, homeodomain-containing transcriptional factors that play a central role in embryonic development and the maintenance of cellular identity crucial to the differentiation of adult stem cells. Implicitly, the pathophysiological relevance of these genes is life-long as evident in many developmental anomalies and adult disorders, particularly cancers. This review considers the normal pattern of HOX gene expression and the signalling pathways involved in its regulation, discusses the consequences of HOX gene dysregulation in developmental anomalies and carcinogenesis, and the plausibility of a common mechanism underlying both processes. Finally, it looks at the potential interventions in cases where cancers are promoted by HOX gene dysregulation.

Keywords: homeobox genes, transcription factors, gene dysregulation, stem cell, carcinogenesis

1. Introduction

Developing a multicellular organism from a single cell is a programmed and finely-tuned process. This is epitomized by the embryonic developmental journey from the single-celled zygote (a totipotent cell) through a complex process of morphogenesis to arrive at a fully developed multicellular organism, which imposes the necessity to organize and position different cell types during development and retain the positional information throughout the organism's life (Grier et al., 2005).

A myriad of genes, transcription factors, and signalling molecules are employed in a timely fashion for this process to deliver the expected outcome. One group of transcription factor-encoding genes, the HOX genes, have captivated the interest of many researchers in the biomedical disciplines over the years because of their crucial role in development. In bilaterian embryos, they play a pivotal role in patterning body structures and the body plan along the head-tail axis (Montavon and Soshnikova, 2014).

The HOX genes are a subset of the homeobox genes family. The latter constitute the second largest transcription factor-encoding genes in the human genome, comprising about 257 genes, 39 of which form the human HOX genes. All homeobox genes contain a well-conserved signature DNA sequence of around 180 base pairs long (Bürglin and Affolter, 2016). This DNA sequence, ubiquitous in homeobox genes, was the reason for the coinage "HOX genes" derived from the contraction of **ho**meobo**x**. However, since it has become clear that HOX genes are not the only ones possessing the homeobox, they are no longer synonymous with homeobox genes (Holland et al., 2007).

The first homeobox was discovered by Walter Gehring in 1983 when he isolated this DNA segment from a mutated gene responsible for a developmental anomaly in Drosophila, where legs grow from the head instead of the antennae (Brinkman, 2014). This phenomenon is referred to as a homeotic transformation (a change of one complete body structure into another). The mutated gene responsible for this phenotype was identified as *antennapedia,* containing a 180-base pair sequence that encodes a DNA binding domain. This sequence is the "homeobox," present in all HOX genes (Holland et al., 2007). Because the transcription factors encoded by HOX genes regulate gene expression and cell differentiation early in embryonic development, directing the differentiation of embryonic stem cells (ESC), mutations in these genes may result in developmental disorders.

HOX genes continue to be expressed throughout adult life. The topographic identity commanded by their expression patterns during development is retained in many adult tissues, particularly in ESC-derived adult stem cells, including hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), epithelial stem cells (EpSCs), and neural stem cells (NSCs) (Dulak *et al*., 2015; Cable *et al*., 2020). This intrinsic positional specificity is maintained during cell differentiation and provides a mechanism for the enduring cell identity and fate restriction in distinct cell types (Steens and Klein, 2022) so that the consequences of aberrations in HOX gene expression are life-long (Lappin *et al.,* 2006)

2. Structural arrangement and functions of HOX genes

The HOX genes in many animal species are organized as clusters, each containing many genes. This pattern is reflected in humans, where the 39 HOX genes are organized in four clusters (A, B, C, and D), mapped at 7p15, 17q21.2, 12q13, and 2q31, respectively (Duboule, 1992).

The first cluster contains genes expressed primarily in the head and neck regions. The genes of the second cluster at 17q21 contain genes whose transcriptional influence is exerted in the thoracic and lumbar regions of the spine. Genes of the third cluster at 12q13 are expressed in the thoracic and lumbar regions and the limbs. The fourth cluster contains genes expressed primarily in the pelvic and tail regions. The orderly expression and regulation of the HOX genes depends on their arrangement, so mutations or rearrangements of the genes can lead to developmental abnormalities and functional disorders, including cancer (Kappen, 2000).

The transcription factors the HOX genes encode bind to specific DNA sequences (enhancers) through which they regulate the expression of downstream target genes, by either repressing or activating the expression of numerous genes. The binding capability is conferred by a 60-amino acid protein, the homeodomain, translated from the 180 base pair homeobox sequence (Brenan, 1989). The homeodomain folds into the helix-turn-helix motif, a structural conformation that is the substructure of many DNA-binding proteins. Each helix-turn-helix consists of two α helices linked by a short strand of amino acids that binds to the major groove of DNA (Brenan, 1989).

Several developmental processes are involved in normal human development, including (1) the migration of cells from primordial sites to where they will ultimately function, (2) the terminal differentiation of precursor cells into specialized cells, (3) the association of groups of cells with similar fates, and (4) the carving of structures and segmental boundaries. As a result, targets of the HOX-encoded transcription factors promote processes such as cell migration, cell division, cell adhesion, and apoptosis, which underlie these developmental processes. In doing so, the HOX genes can act at different hierarchies of development, from the regulation of the entire framework for limb formation, at the top level, to effector genes acting to ultimately form organs, tissues, and other finer details of a given body part (Pearson *et al*., 2005).

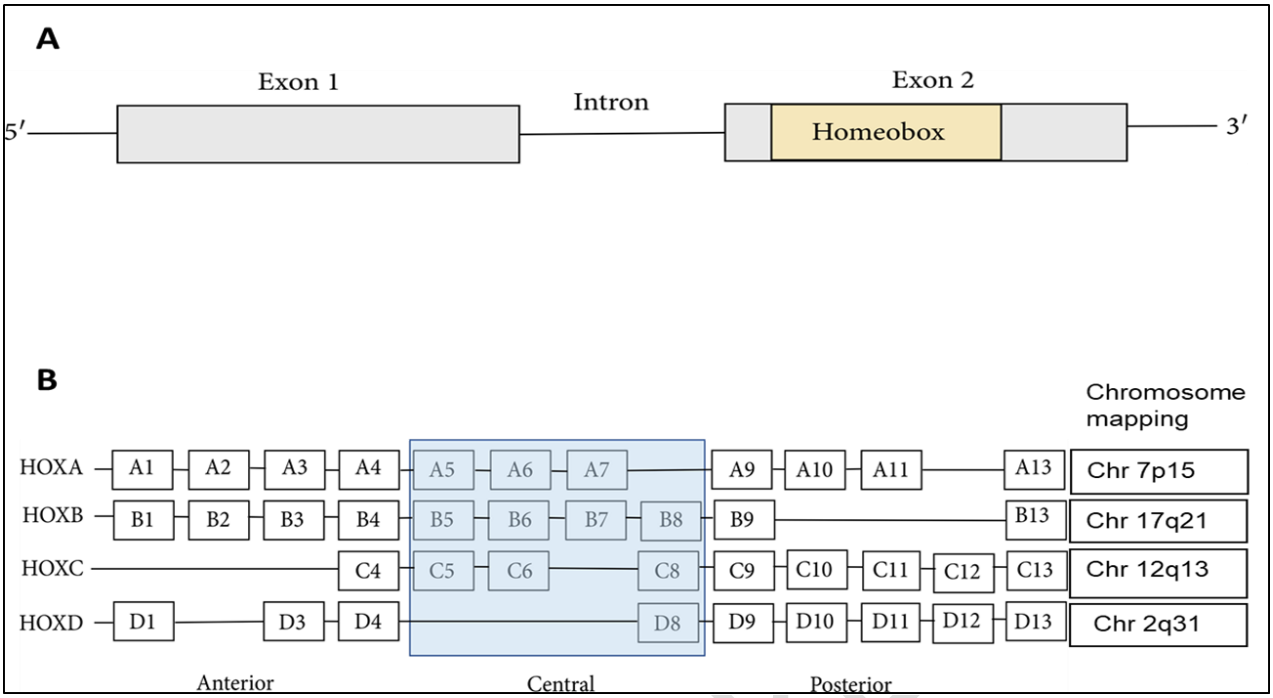

Figure 1. HOX gene structure and genome organization (schematic representation).

(A) HOX genes comprise two exons separated by one intron; exon 2 has a 180-nucleotide sequence (homeobox) encoding the 60 amino acid DNA-binding domain called the homeodomain. (B) The 39 human HOX genes are clustered into the four HOX families, *HOXA*, *HOXB*, *HOXC*, and *HOXD*, with each family consisting of nine to eleven paralogues (related by gene duplication) assigned by numbers based on sequence similarity and cluster positions. These arrangements are responsible for the anterior-posterior specification of body segments. HOX gene expressions exhibit spatial and temporal collinearity: nested domains of HOX genes are generated (the anterior HOX expressions operating earlier in development and posterior HOX expressions occurring later) (Adapted from Bhatlekar et al., 2018, Steens and Klein, 2022)

3. Regulation of Human HOX genes

The critical role of HOX genes in human development necessitates tight control of both the timing and pattern of their expression. This occurs mainly at the transcriptional level, but some regulation at the translational level has been documented (Kondrashov *et al.*, 2011). Transcriptional regulation is achieved within the framework of the clustered organization of the genes, which allows each cluster to share nuclear space and chromatin structure and have common regulatory elements like enhancers and promoters. The timing and the site of HOX gene expression depend on the relative position of the genes within the cluster (Duboule, 2007).

This phenomenon, referred to as collinearity, is both temporal and spatial. In temporal collinearity, the genes are expressed in a time-specific order during embryonic development, while spatial collinearity refers to the expression of the genes in a specific order along the body axis, with the 3' end of the gene cluster being expressed earlier in the anterior (head) region and the 5' end being expressed later in the posterior (tail) region (Mallo and Alonso, 2013).

These highly coordinated expression patterns of HOX genes suggest a degree of global transcriptional regulation of these gene clusters. However, it has been observed that single HOX genes often retain their anterior-posterior expression profile when randomly integrated as transgenes. Furthermore, even the disintegration of the clusters in two or more pieces remains compatible with correct spatial expression patterns during development. The presence of cis-regulatory elements (the enhancers and silencers) close to the HOX genes might explain these observations (Duboule, 2007). The trans-regulators, conversely, constitute gene products, usually proteins, but also miRNAs that bind *cis*-elements to influence the transcription of HOX genes or bind to mRNA to prevent the translation of HOX transcription factors (Li and Jin (2010). There are at least three putative *trans* regulators of HOX genes: retinoic acid (RA), fibroblast growth factors (FGF), and Wnt signalling (Montavon and Soshnikova, 2014).

Evidence suggests that the *Cdx* gene family mediates these signalling pathways, making them the direct regulators of HOX gene expression (Bel-Vialar *et al*., 2002; Wang et al., 2008). The CDX proteins act upstream of HOX genes, relaying signals from RA, FGF, and Wnt canonical pathways to HOX promoters (Lohnes, 2003).

The *Cdx* gene family, also called caudal genes, is a group of three genes in humans, *Cdx1*, *Cdx2*, and *Cdx4*, which play significant roles during development, especially the anterior-posterior patterning of the body (Neijts et al., 2017). However, the exact mechanisms by which Cdx genes regulate HOX gene expression are still not fully understood. More research would be needed to elucidate the molecular interactions between *Cdx* and HOX genes.

3.1 Retinoic acid signalling

Retinoic acid signalling involves cell communication through a vitamin A derivative, retinoic acid. This signalling is mediated by a family of nuclear receptors, namely retinoic acid receptors (RARs) and retinoid X receptors (RXRs), which bind to retinoic acid, and form heterodimers

called retinoic acid response elements (RARE). RARE regulates the expression of target genes (Rhinn and Dolle, 2012) and are found in regulatory regions of many HOX genes (Loring et al., 2005). It is well-documented that hypervitaminosis A causes a range of congenital malformations, including anomalies such as situs inversus, malpositioned hind limbs, spinal bifida, pituitary, and thyroid defects in mammals exposed to abnormally high levels of vitamin A during gestation (Shenefelt, 1972). Human fetuses inadvertently exposed to RA show similar profiles of defects (Lammer *et al*., 1985), and interestingly, the timing and dose of RA exposure are crucial factors in the type and severity of malformations observed in the embryos (Shenefelt, 1972).

Multiple RAREs occur in the cis-acting elements associated with *HOX1* to *HOX5* (Nolte *et al.,* 2013), and when motor neuron cultures are exposed to RA, this group of HOX genes are activated (Mazzoni *et al*., 2013). Even the more "central" genes *(HOX6* to *HOX8)* get activated by RA and tend to be progressively more sensitive with advancing developmental time (Oosterveen *et al.,* 2003). The diverse and significant teratogenic effects of RA suggest its crucial role as a signalling molecule during embryonic development.

3.2 Fibroblast growth factor

Fibroblast growth factors, identified initially as proteins capable of promoting fibroblast proliferation, are made up of 22 cell-signalling proteins produced by macrophages. These exert multiple functions through the binding to and activation of fibroblast growth factor receptors (FGFRs) (Yun *et al*., 2010). FGFs have quite a wide range of effects, which may include regulatory, morphological, and endocrinal functions but commonly mitogenesis (Grieb and Burgess, 2000). In addition, they are promiscuous signalling molecules (many of the different molecules bind to the same receptor to elicit diverse effects) and typically, four receptor subtypes (FGFR1, FGFR2, FGFR3, and FGFR4) are activated by the 22 FGFs to elicit diverse functions like anterior-posterior patterning, mesoderm induction, neural induction, limb development, and neural development, and in mature tissues angiogenesis, keratinocyte organization, and wound healing (Bottcher and Niehrs, 2005). The expression of *HOX6* to *HOX9* is associated with FGF signalling-treatment of chick neural tube cultures with FGF elicits the anterior expression of *HOX6* to *HOX9,* perhaps due to the upregulation of *Cdx* (Van den Akker *et al.,* 2002).

3.3 The Wnt signalling pathway

In humans, the **W**ingless-related i**nt**egration site (WNT) signalling pathways comprise a family of nineteen proteins whose signal transduction regulates a variety of cellular processes, including fate determination, cell migration, cell polarity, neural patterning, and organogenesis during embryonic development (Komiya and Habas, 2008). Mutations affecting this pathway lead to various disorders, including breast and prostate cancer, glioblastoma, and type II diabetes (Komiya and Habas, 2008; Logan and Nusse, 2004).

There are three characterized Wnt signalling pathways: the canonical Wnt pathway, the noncanonical planar cell polarity pathway, and the noncanonical Wnt/calcium pathway. They are all activated by binding a Wnt-protein ligand to a Frizzled family receptor (a transmembrane protein), activating the downstream Dishevelled protein inside the cell. Although most studies suggest that HOX gene regulation occurs primarily by the canonical Wnt signalling pathway, non-canonical Wnt signalling has been shown to also influence *HOX* regulation through crosstalk with the canonical Wnt signalling pathway (Rella *et al*., 2021).

The relationship between aberrant Wnt signalling and disease is quite complex, needing further studies for its elucidation. However, behavioural disorders have been attributed to signalling defects during the early stages of neural development (Mulligan and Cheyette, 2017). Furthermore, aberrant Wnt signalling is also associated with disorders like Alzheimer's disease, glioblastoma, and lung diseases (Arnés and Casas, 2017; Aros et al., 2021).

4. Disorders of HOX genes dysregulation in development and disease

The period of expression of the HOX genes begins from early life, when they direct the differentiation of the ESC during embryogenesis, to later life when they maintain cell identity through ASC-based HOX gene expression. Accordingly, the consequences of HOX gene dysregulation are life-long. Mutations in these genes affect the differentiation of the various categories of stem cells (ESC, ASC, and even the induced pluripotent stem cell [iPSC]) (Kitajima et al., 2016; Bhatlekar et al., 2018). Accordingly, HOX gene aberrations affect embryonic development through the differentiation of ESC and show their influence in adult

pathologies, especially carcinogenesis, through ASC differentiation in later life (Bhatlekar et al., 2016)

4.1. Developmental disorders associated with Embryonic Stem cell

HOX genes-associated developmental disorders derive from germline mutations in the affected genes and are heritable. Reflecting the highly conserved nature of the HOX genes, human phenotypes tend to resemble homologous mouse mutants. In humans, only 10 of the 39 genes, namely *HOXA1, HOXA2, HOXA11, HOXA13, HOXB1, HOXB13, HOXC13, HOXD4, HOXD10, AND HOXD13* have been implicated (Quinonez and Innis, 2014).

The duplication and divergence of ancestral HOX genes in vertebrates have afforded functional redundancy such that each paralogous group, composed of 2 to 4 genes, shares the ability to influence the final phenotype of the embryo. This is shown in studies where a normal or mild phenotype results from single HOX loss-of-function mutations, but a much more severe phenotype follows double or triple knockouts (Wellik, 2009). Accordingly, heterogeneity, variability of penetrance, and expressivity are observed in these disorders (Quinonez and Innis, 2014). Some documented germline mutations in the 10 genes are presented in Table 1.

Table 1 Human HOX gene disorders linked to germline mutations

HOX gene	Clinical syndrome	Molecular genetics/Mutations	References
HOXA1	Bosley–Salih–Alorainy syndrome (BSAS)	BSAS (c.185delG, c.175–176insG and c.84C>G)	Bosley et al.(2008)
	Athabascan brainstem dysgenesis syndrome(ABDS)	ABDS(homozygous c.76CNT)	
HOXA2	Autosomal recessive microtia (a short and narrowed auditory canal, cleft palate, and sometimes unilateral facial paresis)	homozygous mutation (c.556CNA; p.Q186K)	Alasti et al.(2008)
HOXA11	Radioulnar synostosis and thrombocytopenia (RUSAT)	*HOXA11* deletion (c.872delA)- affects the homeodomain of exon 2, resulting in a frameshift and premature translational stop	Thompson & Nguyen(2000)
HOXA13	Hand–foot–genital syndrome(HFGS) and Guttmacher syndrome(GS)	HFGS (*HOXA13* nonsense mutation [c.1107GNA, W369X]- converts a highly conserved tryptophan residue in the homeodomain of *HOXA13* to a stop codon).	Mortlock & Innis (1997),
		GS (missense mutation, c.1112ANT; Q371L)	Guttmacher, A. E. (1993).
HOXB1	Hereditary congenital facial paresis (characterised by congenital facial palsy, hearing loss, strabismus, midface retrusion, and an upturned nose)	homozygous c.619CNT; R207C mutation in *HOXB1*, affecting a highly conserved arginine residue	Webb et al.(2012)
HOXB13	Early-onset prostate cancer	*HOXB13* missense mutation (G84E)- has low penetrance	Ewing et al. (2012)
HOXC13	Hair and nail type ectodermal dysplasia characterised by hypotrichosis and nail dystrophy	Truncating mutations in exon 1 of *HOXC13* (c.390CNA, p.Y130X	Lin et al.(2012)
		27.6 kb deletion involving exon 1 and part of the intron; c.355delC	
		c.200_203dupGCCA, p.H68Qfs*84; c.404CNA, p.S135X)	

HOXD4	Lymphoid malignancy and skeletal malformations (including bilateral cervical ribs and right sacralization of L5)	*HOXD4* missense mutation, c.242A>T; p.E81V	Thim et al.(2005)
HOXD10	Congenital vertical talus (CVT)and Charcot–Marie–Tooth disease(CMT)	heterozygous missense mutation (c.956TNA; M319K) in *HOXD10*	Shrimpton et al. (2004)
HOXD13	Brachydactyly types D and E	Missense mutations (c.947CNG, S316C; c.964ANC, I322L) within the homeodomain of *HOXD13 c*	Goodman et al. (1998)
	Syndactyly type II	Mutations (c.916CNT, R306W; c.683GNT, G228V) within *HOXD13*	Zhao et al. (2007)
	Brachydactyly–syndactyly	Deletion (c.157_177del) in *HOXD13*	Zhao et al. (2007)

4.2 Disorders associated with Adult Stem Cells (ASCs)-Carcinogenesis

Also known as somatic stem cells, ASCs are tissue-specific cells that are precursors of different cell types peculiar to particular tissues and organs. Examples include hematopoietic Stem Cells (HSCs) or hematocytoblasts that generate entire blood cell lineages (Bhatleka, et al., 2018), neural stem cells (NSCs) credited with giving rise to the entire nervous system (Zhao and Moore, 2018), and mesenchymal stem cells (MSCs) capable of differentiating into various mesoderm-derived cells (Figure 2). Interestingly, tumour suppressor genes and oncogenes known to play central roles in carcinogenesis also play essential roles in embryogenesis, which suggests that cellular mechanisms involved in lineage determination and differentiation during development may potentially underlie tumorigenic mechanisms when dysregulated (Feng *et al.,* 2021).

Accordingly, the vast repertoire of stem cells and their descendants create endless tumorigenic potential in the context of HOX gene dysregulation. This is especially relevant in renewing systems with high turnovers such as hematopoietic tissue and gut epithelia. Here, stem cells are

the only ones that persist long enough in the tissue to be able to go through the prolonged sequence of successive mutation and selection requisite for the multistage concept of carcinogenesis (Moolgavkar and Luebeck, 2003). Indeed, abnormalities of HOX gene expression have been observed in many hematological malignancies and solid tumours. The next section of this review focuses on a few examples of HSC and Endodermal Stem cells.

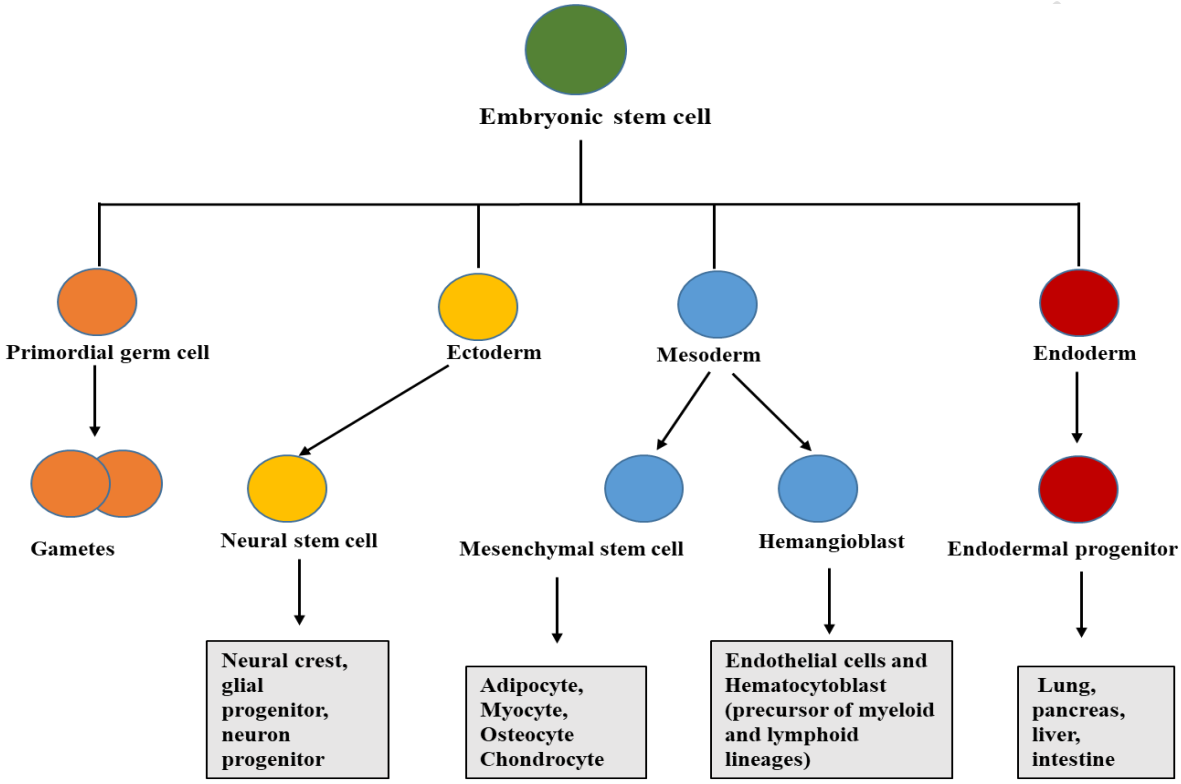

Figure 2. Different lineages of ASCs after differentiation from ESC https://www.cellsignal.com/pathways/stem-cell-and-lineage-markers-pathways?_requestid=1751872&_requestid=2188300

4.2.1. Dysregulation of HOX genes in Hematopoietic stem cells (HSCs)

HSCs are the foundation of hematopoiesis, generating all terminally differentiated and functional hematopoietic lineages. These cells develop from two direct descendants of the HSC namely, the lymphoid progenitor (gives rise to lymphocytes) and myeloid progenitor cell from which neutrophils, basophils, eosinophils, monocytes, and platelets develop (Dzierzak and Speck, 2008).

The expression of Hox genes in hematopoiesis is lineage-specific, and within a given lineage, differentiation-restricted. For instance, in HSC, the expression of *HOXA9*, *HOXB4,* and *HOXB6* regulate self-renewal while *HOXA5* and *HOXA9* expression are involved in HSC proliferation and differentiation to common myeloid progenitors(CMP). *HOXA9* regulates the differentiation of HSC into common lymphoid progenitor (CLP), and *HOXA5* and *HOXC8* are known to be expressed during the erythroid differentiation of megakaryocyte-erythrocyte progenitors (MEP). *HOXC8* play a regulatory role during the differentiation of granulocyte-monocyte progenitor (GMP) cells (Bhatleka, et al., 2018).

Chromosomal translocations that create fusion genes commonly underlie transcriptional perturbation observed in many leukaemia. Changes in HOX gene expression associated with chromosomal translocation have been demonstrated in acute myelogenous leukaemia (AML) (Grier *et al*, 2005). But of particular interest are leukaemias harbouring the mixed lineage leukaemia (MLL) gene mutation. The mutations usually involve the fusion of *MLL* to a partner gene. Over 50 different such partners are known but fusion to *AF4* to produce *MLL-AF4* is the most common (Meyer *et al*, 2013). *MLL* encodes a histone methyltransferase, a transcription factor of HOX genes, which it positively regulates upon direct binding. *MLL* mutations result in hematological malignancies from sustained *HOX* expression and stalled differentiation (Muntean and Hess, 2012). Characteristically, tumours (AML and ALL) harbouring *MLL* mutations are aggressive and associated with poor prognosis (Muntean and Hess, 2012).

4.2.2 Dysregulation of HOX genes in Endodermal Stem Cells

The definitive endoderm forms at gastrulation when epiblast cells migrate through the primitive streak to form a layer beneath the mesoderm. This sheet of endoderm folds to form the primitive gut tube and comprises three portions, the foregut (eventually giving rise to the oesophagus, trachea, lungs, thyroid, parathyroid, thymus, stomach, liver, biliary system and pancreas), the midgut (forming small intestine and part of the colon) and the hindgut, which forms the rest of the colon and upper anal canal (Cheng *et al*., 2013). Various tissue-specific stem cells exist in these histological domains and retain the capacity for self-renewal and tissue-specific

differentiation during which HOX gene expression plays a role. Dysregulation of HOX gene expression in these tissues can promote carcinogenesis.

In the colon, for instance, the colonic crypts formed by the invagination of the lining simple columnar epithelia, house the tissue-specific stem cells at its base, capable of regenerating all intestinal cell types (Humphries and Wright, 2008). *HOXA4, HOXA9,* and *HOXD10* are expressed in normal colonic stem cells during self-renewal and differentiation and dysregulation of these genes produces aberrant stem cells that promote the development of colorectal cancer (CRC). In particular, HOXB9 is known to be an upregulated gene at all stages of CRC development. (Bhatlekar et al., 2014). On the other hand, the loss of expression of *CDX2* and *NKX3.1* in colon cancer represents a case of homeobox genes down-regulation (Grier *et al*., 2004)

4.2.3 Related Therapeutic Prospects

Although the mechanisms underlying many cancers have not been fully elucidated, some therapeutic options, including gene therapy, small molecule inhibitors or RNA interference (RNAi), and epigenetic modification to modulate transcription, are already under consideration

RNAi is being evaluated as an inhibitor of *HOXB7,* a gene-silencing mechanism to ameliorate the growth of prostate cancer cells (Kim et al, 2007). In addition, the RNAi-mediated knockdown of *HOXA10* can be used as a treatment option in multi-drug resistance CML (Yi et al, 2016). Gene therapy targeting the replacement of functional copies of the dysregulated genes has also been explored. In one study, it was shown that overexpression of *HOXA5* inhibits the proliferation and induces the apoptosis of cervical cancer cells (Wang and Wang, 2019)

Finally, epigenetic reprogramming (DNA methylation and histone acetylation) of the HOX genes can also be used to regulate their expression in the therapy of cancers in which HOX dysregulation is a driving force. Such drugs would be inhibitors of DNA-methyltransferases (DNMTs), histone methyltransferases (HMTs), demethylases (HDMs) or deacetylases (HDACs), reversing the epigenetic tags on gene promoters (Paço et al., 2020).

5. Conclusion

The well-timed and orderly pattern of HOX gene expression spanning embryonic development to adulthood is a compelling example of the general dynamism of genetic expression and lays bare the potential consequences of its perturbation. Additionally, the fact that some molecular pathways contribute to both normal development and disease suggests that wellness is maintained within a finely regulated balance. A lot still needs to be elucidated about the full profile of HOX gene functions and regulation, which hopefully, would open new vistas to more intervention targets, especially in the enduring battle against cancer.

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