EDITORIAL

HOX Genes in Pancreatic Development and Cancer

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Summary

The *HOX* genes are a family of homeodomain-containing transcription factors that determine cellular identity during development and which are subsequently re-expressed in many types of cancer. Some recent studies have shown that *HOX* genes may have key roles both in pancreatic development and in adult diseases of the pancreas, including cancer. In this review we consider recent advances in elucidating the role of *HOX* genes in these processes, how they may connect early developmental events to subsequent adult disease, and their potential both as diagnostic markers and therapeutic targets.

HOX Genes

The development and maintenance of cellular identity is vital in both embryonic and adult tissues for normal organ function. Key to this is the establishment of stable transcriptional states within the cell, a process in which transcription factors have a key role. One group of transcription factors of particular note in this regard are the *HOX* genes, a family of homeodomain-containing transcription factors that determine cellular and tissue identity by regulating specific transcriptional programmes [1, 2, 3]. Mammals have 39 *HOX* genes split between four groups of linked genes on different chromosomes, which are thought to have arisen from a series of duplication events. These groups are known as A, B, C and D, and the genes within each group are numbered from the 3' most member (1) to the 5' most member (13) [4]. Thus, for example the 3' most member of the *HOXA* group is known as *HOXA1* (Figure 1). Equivalently numbered genes in each group (e.g., *HOXB4* and *HOXD4*) are referred to as paralogues and are thought to have arisen from a common ancestral gene as they represent the same position within the ancestral *HOX* cluster. Members of each group often share enhancer regions and are co-regulated, giving rise, in part, to coordinated temporal and spatial expression patterns in the developing embryo whereby the 3' most *HOX* genes are expressed earlier and more anteriorly than their more 5' neighbours [5]. In this manner the *HOX* genes give rise to a pattern of overlapping expression domains along the anteroposterior axis that is key to defining the identities of cells along it [5].

The HOX transcription factors have a relatively limited specificity for binding to DNA, but this is enhanced by the binding of co-factors such as PBX and MEIS that increase DNA binding specificity and also modify transcriptional regulation [6, 7, 8]. These co-factors, as well as the *HOX* genes themselves, are highly conserved between animal phyla. The *HOX* genes also show considerable similarities between each other, especially paralogues and neighbouring members of the same group [1, 2, 3]. This has resulted in a high degree of functional redundancy, particular in respect to early developmental events although later, more organ specific development is generally dependent on a smaller number of *HOX* genes [9, 10]. The expression domains of *HOX* genes that are established during development are generally preserved in the adult [11], and there are a number of examples where adult *HOX* expression is required for the continuation of correct cell identities. This is most apparent where cells are turning over quickly, for example in the proliferation and differentiation of blood cells [1] and in the renewal

Key words HOXB7 protein, human; HOXD13 protein, human; HXR9 peptide; Pancreatic Neoplasms

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Document URL http://www.joplink.net/prev/201105/01.html

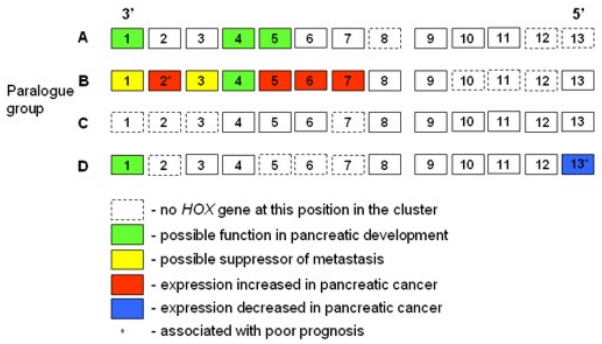

Figure 1. The arrangement of *HOX* genes in the mammalian genome and their potential roles in pancreatic development and cancer.

of the endometrium [12]. The importance of *HOX* genes in these processes indicates a role in the promotion of cell proliferation and survival, in addition to maintaining cellular identity, and indeed *HOXB4* is crucial for the continued proliferation of hematopoietic stem cells [1]. It is perhaps not surprising then that *HOX* genes are frequently deregulated in cancer, where their primary function also seems to be in promoting proliferation whilst preventing apoptosis [13, 14, 15, 16, 17].

HOX Genes in Pancreas Development

The pancreas develops from endodermal cells in the future midgut region of the embryo, and is dependant on a number of inductive interactions between the endoderm and other tissues. The best characterised of these events is the development of the dorsal pancreatic primordia which is initiated in the endoderm by signalling from the overlying notochord through the secreted proteins activin and FGF-2. These signals are not spatially restricted to the notochord adjacent to the prepancreatic endoderm however, and the responsiveness of endodermal cells is presumably modified by pre-existing anteroposterior information in the endoderm. The *HOX* genes are key determinates of anteroposterior identity [2, 5, 18], and the expression patterns of *HOX* genes in the early endoderm suggests that *HOXA4*, *HOXA5* and *HOXB4* provide the spatial information needed to restrict the response to signals from the notochord [19]. Correspondingly, retinoic acid, which regulates *HOX* expression through binding to nuclear hormone receptors, is also known to have a key role in pancreatic development [20]. Furthermore retinoic acid is sufficient to drive embryonic stem cell differentiation towards functional insulin producing cells [21], and *HOXA4*, *HOXA5*, *HOXB4* and *HOXA1* (see below) are all activated directly by retinoic acid [22].

These early patterning events give rise to pancreatic progenitor cells which, in turn, are subdivided into exocrine and endocrine progenitors through the presence or absence of Notch signalling, respectively. In a number of early developmental processes Notch signalling is dependent on HOX transcription factors, together with the PBX co-factor [18, 23, 24], with the HOX1 paralogues (*HOXA1* and *HOXD1*) mediating key transcriptional changes [18, 24]. Likewise *HOXA1* expression is activated in pancreatic exocrine cells and is required for exocrine development, possibly by modulating TGF-beta signalling from the foregut mesoderm [25].

The endocrine progenitor cells are also defined by the expression of specific transcription factors, *Pax-6* for alpha-cells and gamma-cells (glucagon and pancreatic polypeptide secreting, respectively), and *Pax-4* for beta-cells and delta-cells (insulin and somatostatin secreting, respectively). *Pax* transcription factors are known to cross-regulate *HOX* target genes, at least in part through direct interactions with the latter [26], and also through the direct regulation of a number of *HOX* genes including *HOXD4* [27]. Thus, *HOX* genes play a number of key roles in pancreatic development from the specification of early endodermal progenitor cells through to the determination of specific cellular subtypes within the maturing pancreas.

HOX Expression and Function in Pancreatic Cancer

The deregulation of *HOX* genes in cancer is now well established, although in general rather less is known about their function [28]. For a number of malignancies, including melanoma [14], myeloma [13], and ovarian [15], renal [17], lung [16] and, indeed, pancreatic cancer [29], it has been shown that the *HOX* genes of paralogue groups 1 through 9 can promote cell survival by blocking apoptosis. In this respect many of these 27 *HOX* genes have a redundant or at least a highly overlapping function [13, 17, 29]. Targeting the anti-apoptotic function of this group of genes has been achieved by antagonising the interaction between HOX proteins and the PBX co-factor. This approach exploits a highly conserved hexapeptide motif on PBX that is required for HOX binding [6, 7, 8]. The motif forms part of peptides such as HXR9 that act as competitive antagonists of HOX/PBX dimer formation can induce apoptosis both *in vitro* and *in vivo*, at least in part through the greatly elevated expression of *cFos* [13, 17, 29]. Disrupting HOX/PBX dimer formation in the pancreatic cancer derived cell line T3M4 also blocks cell proliferation [29], and reduces the expression of a number of cancer-related target genes by at least one order of magnitude. These include *TMPRSS3*, a transmembrane serine protease involved in tumour invasion and metastasis that is frequently over expressed in pancreatic cancer [30], and the S100 calcium binding protein P (S100P) which is involved in regulating the cell cycle and cell proliferation [31]. Interestingly *HOXB2* and *HOXA10* are also down regulated when HOX/PBX dimer formation is blocked, indicating a possible auto-regulatory pathway for these genes [29].

Other functions of *HOX* genes in pancreatic cancer may be more specific to particular members of the *HOX* family. Although to date there is only very limited data on *HOX* expression in normal pancreatic tissue of both the developing and adult pancreas, it would seem that generally these genes (*HOXA1* [25], *HOXA4* [19], *HOXA5* [19], and *HOXB4* [19]) are not up regulated in pancreatic cancer. Instead, other *HOX* genes are expressed, some of which have known oncogenic functions. Most notable amongst these is *HOXB7* which has been shown to mediate epithelial to mesenchymal transition in breast cancer cells through the induction of *bFGF* [32], and to promote proliferation in oral cancer [33] and progression and metastasis in lung cancer [34]. *HOXB7* is also over expressed in pancreatic cancer, both in primary pancreatic adenocarcinoma and in the pancreatic adenocarcinoma cells lines AsPc-1, BxPC-3, MiaPACA and PANC1 [35]. Two neighbouring genes

of the *HOXB* cluster, *HOXB5* and *HOXB6*, are also over-expressed in pancreatic cell lines as well as resected infiltrating pancreatic cancer tissue. Although the significance of this finding is unknown, it is noteworthy that forced over expression of *HOXB6* in murine bone marrow is sufficient to immortalise a population of myelomonocytic precursor cells leading to acute myeloid leukemia [36]. Also up regulated in pancreatic cancer is *HOXB2* [37], which was found to be present in 38% of pancreatic tumours. *HOXB2* was shown to have prognostic value, as its expression is associated with nonresectable tumours, and when presented in resected tumours it is associated with poor survival. This finding may relate to the apparent ability of *HOXB2* to promote metastasis in lung cancer, where its expression is also associated with a poor prognosis [38]. *HOXB2* additionally promotes proliferation, as it is bound by the tumour suppressor protein p205 that is known to delay G2/M progression in dividing cells [39].

Other *HOX* genes are strongly down-regulated in pancreatic cancer. These include *HOXD13*, which is originally involved in the determination of the terminal digestive and urogenital tracts. *HOXD13* expression is lower in tumour tissue compared to normal pancreatic parenchyma [40], and the absence of *HOXD13* expression in tumours is associated with a significantly poorer prognosis, the 12-month survival rate for patients with *HOXD13*- tumours being 17.2% as compared to 79.8% for patients with *HOXD13*+ tumours. These findings imply that *HOXD13* functions as a suppressor of metastasis, a characteristic shared by two further *HOX* genes, *HOXB1* and *HOXB3* [41]. Specific knockdown of either of these genes is sufficient to reduce the migration of pancreatic cancer cells *in vitro* and invasion and metastasis of pancreatic cancer *in vivo*, using zebrafish embryo xenotransplantation models. The same study also showed that *HOXB1* and *HOXB3* are suppressed by the microRNA *miR-10a*, the expression of which is significantly higher in metastatic pancreatic cancer [41].

HOX gene deregulation is also associated with pre-malignant pancreatic intraepithelial neoplasia (PanIN). *HOXB2* was found to be expressed in 15% of early PanIN lesions [37], and as described above, is also associated with the fully malignant state, it is therefore possible that expression of *HOXB2* in PanIN lesions may predict the development of cancer but this is yet to be shown conclusively. Also up regulated in PanIN is *HOXA5* [42], which is of interest because other studies have suggested that its function is closer to a tumour suppressor rather than an oncogene as it activates the transcription of a number of key tumour suppressors, including *p53* [43].

Future Directions

Although relatively little is still known of *HOX* expression and function in pancreatic cancer as compared to many other cancer types, it is already clear that *HOX* genes are of functional significance to the malignant phenotype. It is not therefore surprising to find that a number of *HOX* genes have prognostic value and may ultimately be of clinical relevance [38, 40]. More importantly still, *HOX* gene expression may prove to have diagnostic value for pancreatic cancer. The extremely poor survival rates for this disease are due in part to its predominantly late diagnosis; specific *HOX* genes expressed in, for example, circulating tumour cells, could potentially detect the presence of pancreatic cancer before the onset of symptoms. Additionally, a similar approach might also be of value in defining pancreatic cancer as a primary tumour in cases where the origin of metastasis is uncertain.

HOX genes are also potential therapeutic targets in pancreatic cancer. To date the only successful strategy for blocking *HOX* gene function is through interfering with the HOX/PBX interaction using a peptide mimic (HXR9) of the conserved PBX binding domain in HOX [13, 14, 15, 16, 17]. This is a novel strategy in pancreatic cancer, and is worthy of investigation as a possible therapeutic approach.

Conflict of interest The authors have no potential conflict of interest

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