DEREGULATED HOMEBOX GENE EXPRESSION IN CANCER: CAUSE OR CONSEQUENCE?

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Homebox genes comprise a large and essential family of developmental regulators that are vital for all aspects of growth and differentiation. Although many studies have reported their deregulated expression in cancer, few studies have established direct functional roles for homebox genes in carcinogenesis. Nonetheless, most cases of deregulated homebox gene expression in cancer conform to a simple rule: those that are normally expressed in undifferentiated cells are upregulated in cancer, whereas those that are normally expressed in differentiated tissues are downregulated in cancer.

It is widely accepted that many of the molecular pathways that underlie carcinogenesis represent aberrations of the normal processes that control embryogenesis. There are many examples in which the anomalous expression of genes that regulate growth and development have been implicated in carcinogenesis. Among the principal examples are homeobox genes, which encode transcriptional regulatory proteins (homeoproteins) that are widely used during development and are often aberrantly expressed in cancer (Box 1). Based on their global significance for development and differentiation, and their frequent deregulation in cancer, homeobox genes provide an ideal focal point to explore the intimate relationship between oncogenesis and embryogenesis.

How are homeobox genes deregulated in cancer, and what are the potential consequences for carcinogenesis? It was originally thought that homeobox genes were transcriptional activators that promote oncogenesis through their aberrant upregulation in carcinoma cells. However, it now seems that the actual scenario is far more complex, as both losses and gains of homeobox gene expression are associated with tumorigenesis, and there is no obvious relationship between the transcriptional properties of homeoproteins and their oncogenic potential.

Homebox genes are developmental regulators
The homebox was first identified in the 1980s as a sequence motif that was shared among Drosophila homeotic genes (the HOM-C complex), and is now known to be present in many genes in virtually all eukaryotic species. The HOM-C genes are still considered to be the prototype homebox genes, and their counterparts in humans and mice — the HOX complexes (HOX–D clusters) — are among the most widely studied vertebrate homebox genes (Box 2). Nonetheless, the HOM-C/HOX clusters represent only a subset of all known or predicted homebox genes. It is estimated that the human genome, for example, includes at least 200 homeobox genes, of which only 39 are members of the HOX family. Unlike the HOM-C/HOX genes, which are organized on chromosome clusters, most homebox genes are dispersed throughout the genome.

Homebox genes are divided into families (and subfamilies) on the basis of the level of similarity among their respective homeodomains (Box 3). These families vary in size from the relatively large ones, such as the HOX group, that comprises 39 members, to the small families, such as the MSX and Engrailed (EN) groups, which only have two or three members each. Most homebox gene families, such as the NKX, PAX and DLX groups, are intermediate in size and contain five to
HOMEOSTIC GENES
Mutations of these Drosophila genes result in homeotic mutations — the conversion of one body part to another. Homeotic genes were identified as part of a hierarchy of genes that control Drosophila development. Other classes of genes in this hierarchy, such as the gap, pair-rule and segment polarity genes, also include homeobox genes.
HOMEODOMAIN
The 60-amino-acid protein domain encoded by the homeobox.
PARALOGOUS HOX GENES
Refers to genes located in the same position on the four Hox clusters. In general, paralogous Hox genes (for example HoxA8 and HoxB9) are more similar to each other than to adjacent genes on the same Hox cluster (for example, HoxA9 and HoxA10).
EPITHELIAL–MIESCHYMAI INTERACTIONS
Describe the reciprocal signalling between epithelial and mesenchymal tissue layers during embryogenesis. Epithelial–mesenchymal interactions are essential for the formation of many organs.
ALVEOLAR RHADOMYSARCOMA
A soft-tissue tumour with muscle differentiation occurring in children.

### Summary
- Although deregulated homebox gene expression was originally associated with oncogenic activities, it is now apparent that homeobox genes might be lost as well as gained in cancer, and their corresponding activities might be tumour suppressing as well as tumour promoting. Although there are numerous examples of the deregulated expression of homeobox genes in association with cancer, there are far fewer cases of causal links between these aberrant expressions and carcinogenesis.
- Many examples of deregulated homebox gene expression in cancer conform to a simple rule: homeobox genes that are upregulated in cancer are normally expressed during development and/or in undifferentiated cells, whereas homeobox genes that are downregulated in cancer are normally expressed in adulthood and/or in differentiated tissues.
- Several examples exist in which homeobox genes impinge on the cell cycle, indicating that deregulation of cell-cycle control might be a common mechanism by which aberrant homeobox gene expression contributes to carcinogenesis.
- Homeobox genes that are downregulated in cancer share several features, including tissue specificity and epigenetic loss of function.
- Based on their unique features with respect to their expression and function, homeobox genes are best described as ‘tumour modulators’ rather than oncogenes or tumour-suppressor genes.

**Box 1** Derivation of homeobox genes through translocations

In addition to their deregulated expression in solid tumours, homeobox genes are frequently translocated in leukaemia and sarcomas.

In sarcomas — such as paediatric alveolar rhabdomyosarcoma — PAX3 and PAX7 are translocated, generating a fusion protein that contains the DNA-binding regions of PAX3 (or PAX7) fused to the transcriptional-activation domains of the forkhead transcription factor. As the chimeric fusion protein is a potent transcriptional activator, its transforming potential is presumed to be due to the inappropriate activation of target genes (REFS 76–81).

In leukaemia, HoxA9 and other Hox genes are involved in translocations that result from fusion proteins with a nucleoporin protein, NUP98. The fusion proteins, which contain the DNA-binding domains of these Hox proteins and the amino-terminal region of NUP98, inhibit differentiation and promote transformation of haematopoietic progenitor cells. Derearrangement of HoxA9 in myeloid leukaemia is strongly correlated with MIEIS1, which is related to the PBX homeobox family. Notably, PBX1 was identified on the basis of its transformation in B-cell leukaemia, which results in a chimeric fusion protein (E2A–PBX1) that contains most of PBX1 fused to the 5’ region of E2A. As protein–protein interactions between members of the PBX/MIEIS and Hox families result in cooperative DNA binding, it has been postulated that the cooperativity of these homeobox genes in promoting tumorigenicity of haematopoietic cells reflects their synergistic biochemical properties (REFS 82–87).

**Homeoproteins are transcriptional regulators**
The homeodomain mediates sequence-specific interactions with DNA elements, primarily those that contain a TAAT core motif (BOX 3). Accordingly, many homeoproteins have been shown to function as transcriptional regulators — some as activators and others as repressors. Nonetheless, there are relatively few examples in which bona fide target genes have been identified and are regulated by specific homeobox genes in vivo. One of the main limitations in identifying target genes has been reconciling the relatively promiscuous DNA-binding specificities that homeoproteins display in vitro with their highly selective functions in vivo. It is now apparent that the functional specificities of homeoproteins are dictated by several tiers of regulation, including post-transcriptional controls, nuclear–cytoplasmic transport and protein–protein interactions (FIG. 1).
Box 2 | The mystery of the HOM-C/HOX gene clusters

A striking feature of the HOM-C/HOX families is their conservation not only at the sequence level, but also at the chromosome level, because members of these gene families are linked on chromosome clusters. Whereas Drosophila has a single HOM-C cluster, humans (and mice) have four HOX genes clusters (A–D), which seem to have arisen by gene duplication. Another conserved feature is the collinear expression of the HOM-C and HOX genes. Genes that are located towards the 5’ end on the chromosome are expressed more posteriorly in the embryo, whereas those more 3’ are expressed more anteriorly (see figure). This collinearity of chromosome structure and embryonic expression is conserved throughout evolution, although its functional significance is unresolved. Recent studies have indicated that this collinearity in the PARAXIAL MESODERM is related to the sequential activation of HOX genes that are regulated by the segmentation clock, which controls the pace at which segmental boundaries are formed.

Drosophila

Mouse embryo

One example is the interaction between the HOX and MEIS/PBX families, the members of which form protein complexes in vitro and display cooperative interactions with DNA. In addition to promoting DNA-binding specificity and affinity, protein–protein interactions might also regulate nuclear–cytoplasmic transport, as shown for certain Drosophila homeoproteins. Notably, these biochemical activities contribute to the oncogenic potentials of these homeobox genes, as HOX and PBX proteins also cooperate in cellular transformation.

Although it is widely accepted that the binding promiscuity of homeoproteins in vitro reflects their requirement for co-factors (as described above), a complementary view is that the widespread DNA binding of homeoproteins might reflect their actual function as regulators of many gene targets simultaneously. The advent of chromatin immunoprecipitation, combined with microarray profiling, could provide real insights into target genes that are regulated by homeoproteins, as well as the molecular bases for specificity.

Aberrant expression of homeobox genes in cancer

Following the initial reports that HOX genes are expressed in carcinoma cell lines, as well as the corresponding embryonic tissues from which these tumour cells are derived (for example, refs 27, 28; reviewed in ref. 29), the deregulated expression of homeobox genes has been described in many solid tumours and derivative cell lines (Table 1). These expressions can be considered in three broad categories.

First, homeobox genes can be re-expressed in the tumorigenic cells that are derivatives of embryonic cells in which the particular homeobox gene is normally expressed during development. There are many examples, including brain, mammary gland and kidney, in which the HOX genes are expressed in tumours that are derived from tissues in which the HOX genes are normally expressed during development. Most cases of deregulated homeobox genes in cancer fall into this category (Table 1; Online Table 1).

Second, homeobox genes can be expressed in tumorigenic cells derived from those in which a particular homeobox gene is not normally expressed during development. For example, PAX5 is expressed in medulloblastoma, but not in the cerebellum from which this tumour is derived. Given the relatively few examples in this category, it is possible that the ‘new’ expression of homeobox genes in cancer — as opposed to their ‘re-expression’ — could be the exception rather than the rule.

Third, homeobox genes can be downregulated in tumorigenic cells that are derived from tissues in which a particular homeobox gene is normally expressed in the fully differentiated state. Examples include the loss of expression of CDX2 in colon cancer and NKX3.1 in prostate cancer. Although there are fewer examples in this category, at present, the concept that homeobox genes can be downregulated (and not always upregulated) in cancer is relatively new, with other examples yet to be described.

Typically, those homeobox genes that are upregulated or ‘gained’ in carcinoma (categories 1 and 2) normally show expression patterns that are restricted to undifferentiated or proliferative cells. Conversely, homeobox genes that are downregulated or ‘lost’ in carcinoma (category 3) are normally expressed in fully differentiated tissues. It is striking how these broad generalizations so accurately recapitulate the examples in Table 1 (and Online Table 1). For example, HOX and MSX genes, which are normally expressed in undifferentiated tissues, are typically associated with ‘gains’ of expression in carcinoma, whereas CDX and NKX genes, which are expressed in fully differentiated tissues, are associated with a ‘loss’ of expression in cancer (Table 1). In broad

PARAXIAL MESODERM
The tissue that gives rise to the somites during development.
Despite numerous reports of aberrant homebox gene expression in solid tumours (Table 1. Online Table 1), our current knowledge is far from complete. Assuming that their altered expression promotes the cancer phenotype, a comprehensive survey of homebox gene expression in tumour specimens might provide insight into the potential specificities of particular homebox genes or families of genes for particular tumour types, oncogenic stages or specific cell types. Although HOX genes clearly dominate the literature of deregulated homebox genes in cancer (Table 1. Online Table 1), it is not at all apparent whether this reflects their particular significance for carcinogenesis or the fact that they have been studied more extensively than other classes of homebox genes. It is more likely that the answer lies between these extremes and, undoubtedly, a comprehensive analysis of the expression patterns of homebox genes in solid tumours is warranted.

**Gain of homebox gene expression in carcinoma**

In support of their causal role in carcinogenesis, gain of function of certain classes of homebox genes has been shown to promote the oncogenic phenotype in cell culture models. Misexpression of HOX genes in non-tumorigenic fibroblast cell lines results in increased cellular proliferation, tumorigenicity in cell culture and tumour growth when cells are injected into nude mice. Notably, PAX genes, including...
those that lack a homeobox, show similar results\(^5\), so such activity might not be solely dependent on the homeobox. Members of the MSX gene family also show transforming activities if misexpressed in non-tumorigenic cells\(^11,36\), and it has been suggested that these genes are downstream targets of RAS signalling in tumour cell lines\(^36\).

It has been presumed — although not necessarily proven — that these oncogenic activities are produced by wild-type, rather than mutant, homeoproteins. The implication is that the oncogenic potential of homeoproteins is not due to the acquisition of new or alternative activities, but is, instead, a byproduct of their normal functions occurring in the wrong cellular context. For example, the overexpression of \textit{HOXB7} has been shown to activate \textit{BASIC FIBROBLAST GROWTH FACTOR} (bFGF), which promotes cellular proliferation\(^38,39\). It has been proposed that bFGF might represent a normal target of HOX gene activation during embryogenesis and, therefore, the aberrant expression of HOX genes in cancer cells leads to the inappropriate activation of bFGF, which, in turn, promotes cell proliferation.

There are now several examples in which the oncogenic activities of particular homeoproteins have been attributed to their inappropriate effects on cell-cycle regulation, and these are thought to reflect an erroneous extension of their normal embryonic functions (Fig. 2). For example, based on the correlation between the ability of MSX genes to inhibit differentiation and the upregulation of \textit{cyclin D1} (REF 15) — which is involved in regulating the G1–S transition — a current model proposes that MSX genes block terminal differentiation during embryogenesis by preventing cells from exiting the cell cycle. Therefore, the transforming activities of MSX genes in cancer cells might reflect their inappropriate upregulation of cyclin D1, which promotes or maintains an undifferentiated state. Owing to the elevated levels of cyclin D1 in mammary carcinogenesis\(^46\), the link between MSX genes and cyclin D1 in the mammary gland\(^13\) is of particular interest. Although MSX genes might be deregulated in many types of \textit{epithelial cancer}\(^41\), it is conceivable that their causal effects for carcinogenesis are more restricted, and perhaps limited to tumour types for which their downstream target genes, such as cyclin D1, are of primary significance.

\textit{HSIX1} is another homeoprotein for which the oncogenic activities seem to be mediated by inappropriate control of the cell cycle. \textit{HSIX1} is the human homologue of the \textit{Drosophila Six} gene that was originally isolated on the basis of its enriched expression during S phase\(^41\). Its misexpression in cell culture does not significantly affect proliferation; however, HSIX1 abrogates the DNA-damage-induced G2 cell-cycle checkpoint\(^42,43\). Notably, HSIX1 is upregulated in primary and metastatic \textit{breast cancer}, as well as in other tumour types\(^42\), raising the possibility that its activities in cell culture are relevant for tumorigenesis \textit{in vivo}. HOX11 (which is not a member of the HOX gene family) has also been implicated in controlling the G2 cell-cycle checkpoint through its interactions with the protein phosphatases (PP2A and PP1) that regulate the transition to M phase\(^44\). HOX11 can bind to the catalytic subunit of these phosphatases, thereby disrupting a G2 checkpoint and allowing cells to proceed inappropriately through M phase. This home-box gene is localized to a recurrent chromosomal breakpoint in T-cell acute leukaemia\(^44,45\), and is normally expressed during haematopoiesis and is required for spleen formation\(^46\). These findings — linking \textit{HSIX1} and \textit{HOX11} to the regulation of the G2 checkpoint — raise the intriguing possibility that deregulation of homeobox genes in cancer might have adverse effects on the maintenance of genomic stability by allowing cells to proceed inappropriately past the DNA-damage-induced checkpoint.

So, the available evidence supports the general conclusion that 'gains' of expression of certain homeobox genes in cancer cells are oncogenic, and that these oncogenic activities represent an inappropriate display of their normal functions during embryogenesis. This implies a certain degree of selectivity between particular homeoproteins and the tumour types in which their functional consequences are manifest. In other words, the functional outcome for oncogenesis might vary depending on the cell type in which the particular homeobox gene is expressed. Indeed, there is at least one example in which the loss (rather than gain) of function of a HOX gene (\textit{HOXA5}) has been implicated in breast carcinogenesis; in particular, methylation of the \textit{HOXA5} promoter and a concomitant loss of HOXA5 protein expression were correlated with loss of p53 expression\(^48\).
Table 1 | Deregulated homebox genes in solid tumours

<table>
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<tr>
<th>Genes</th>
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<th>Normal expression</th>
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<tr>
<td>HOX*</td>
<td>Gain of expression in primary tumours and cell lines from brain, breast, colon, lung and kidney</td>
<td>Expression patterns during embryogenesis reflect roles in patterning, segmentation and fate determination of many tissues</td>
<td>Overexpression promotes cellular transformation in culture</td>
<td>27,28,33, 34,91–93</td>
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<tr>
<td>MSX (1,2)</td>
<td>Gain of expression in mammary, colon, stomach, kidney, thyroid and other carcinomas</td>
<td>Expression during embryogenesis associated with epithelial-mesenchymal interactions and inversely correlated with differentiation</td>
<td>Overexpression leads to inhibition of differentiation, correlated with upregulation of cyclin D1</td>
<td>11,12,15, 41</td>
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<tr>
<td>HSX1</td>
<td>Gain of expression in mammary and other carcinomas</td>
<td>Limited expression analyses available; homologues expressed in the developing brain, eye, muscle and other tissues</td>
<td>Overexpression abrogates the G2 cell-cycle checkpoint in response to X-ray irradiation</td>
<td>43,94</td>
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<td>GBX2</td>
<td>Gain of expression in prostate carcinoma</td>
<td>Expressed in the developing nervous system; limited information concerning expression in prostate</td>
<td>Downregulation of GBX2 via antisense correlated with reduced tumorigenicity</td>
<td>95–97</td>
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<td>PAX (2,5,6,8)</td>
<td>Gain of expression in Wilms’ tumour, brain and breast cancer; translocation of PAX8 in thyroid carcinoma</td>
<td>Expression patterns during embryogenesis reflect roles in organogenesis of kidney and other tissues</td>
<td>Overexpression promotes cellular transformation in culture</td>
<td>30,35, 98–101</td>
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<tr>
<td>CDX2</td>
<td>Loss of protein expression in colon carcinoma</td>
<td>Expressed during embryogenesis in extraembryonic and embryonic tissues; expression in older embryos and adults restricted to intestinal epithelium</td>
<td>Overexpression promotes differentiation of intestinal cells, while leading to reduced proliferation and tumorigenicity; heterozygous mutant mice are predisposed to colon cancer</td>
<td>32,57–59</td>
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<tr>
<td>NKX3.1</td>
<td>Loss of protein expression in prostate cancer and pre-neoplastic (PIN) lesions</td>
<td>Expressed during embryogenesis in somites and other derivatives; expression in older embryos and adults is restricted to prostatic epithelium</td>
<td>Localized to 8p21, which is frequently deleted in prostate cancer; overexpression leads to reduced cell growth and tumorigenicity; homozygous and heterozygous mutant mice are predisposed to prostate cancer</td>
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<tr>
<td>BARX2</td>
<td>Loss of expression in ovary carcinoma</td>
<td>Expressed in normal ovarian surface epithelium; limited expression analyses available</td>
<td>Localized to 11q24, which is frequently deleted in ovarian cancer; displays tumour suppression and anti-metastatic activities in cell culture</td>
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Table contains selected examples and references; a comprehensive summary of deregulated homebox genes in solid tumours and complete reference list is provided as supplementary material (see ONLINE TABLE I). *Refers to members of the HOX–D clusters, most of which have been reported to be deregulated in cancer.

Moreover, not all homeoproteins promote proliferation; for example, the forced expression of the GAX1 homebox gene represses cellular proliferation by upregulating CDKN1A, which encodes the cyclin-dependent kinase inhibitor WAF1 (also known as p21)31. So, although it is clear that certain classes of homebox genes have oncogenic properties, many issues regarding their precise functions in carcinogenesis of specific tumour types remain to be explored.

Loss of homebox gene expression in carcinoma

Until recently, the upregulation of homebox genes in cancer has been equated with oncogenesis, as it had not been appreciated that some classes of homebox genes have inhibitory consequences for tumorigenesis. However, there are now several, well-characterized examples in which the loss of function of homebox genes has been implicated in tumorigenesis. These genes — namely CDX2 and NKX3.1 — are members of homebox families that are expressed in fully differentiated tissues, and the expression of which is maintained for differentiation and/or function of these tissues.

CDX2 is normally expressed in the gut during development and adulthood32–34; however, its expression is lost in colorectal tumours and corresponding carcinoma cell lines35,36. A functional role for the loss of function of Cdx2 in colorectal carcinoma has been indicated by the frequent occurrence of adenomatous intestinal polyps in Cdx2 heterozygous mutant mice37; notably, these precursor lesions do not progress to overt carcinoma, even in aged mice. The misexpression of Cdx2 in colorectal cell lines inhibits cell growth and tumorigenicity38, whereas it promotes differentiation of non-tumorigenic cells39.
The wild-type allele remains intact in the pre-malignant lesions from Cdx2 heterozygous mutant mice, despite the loss of Cdx2 protein expression\(^{77,80}\). This parallels the situation in human colorectal cancer in which Cdx2 is rarely mutated\(^{66,68}\), although its protein expression is frequently lost\(^{53}\). Therefore, Cdx2 seems to be genetically haploinsufficient, as the loss of a single allele produces the cancer-prone phenotype. However, genetic inactivation of Cdx2 does not seem to be absolutely required for its loss of function, as pre-malignant colon lesions in Min mutant mice, which have two wild-type Cdx2 alleles, have also lost Cdx2 protein expression\(^{51}\). So, epigenetic inactivation of Cdx2 by loss of protein expression could be a common event that predisposes to, but is not sufficient for, colorectal cancer.

There are several striking parallels between the role of CDX2 in colon cancer and that of NKX3.1 in prostate cancer. Analogously to CDX2, NKX3.1 is expressed from the earliest stages of prostate formation to adulthood and is required for differentiation of the prostatic epithelium\(^{66-68,69,70}\). Homozygous and heterozygous Nkx3.1 mutant mice develop pre-cancerous lesions of the prostatic epithelium, termed PROSTATIC INTRAEPITHELIAL NEOPLASIA (PIN)\(^{66-68,70}\). Although these PIN lesions do not progress to carcinoma, even in aged Nkx3.1 mutants, loss of function of Nkx3.1 enhances prostate cancer progression in collaboration with loss of function of the Pten tumour-suppressor gene\(^{69}\). Furthermore, the prostatic epithelium of Nkx3.1-mutant mice shows increased proliferative activity\(^{70}\), whereas overexpression of Nkx3.1 in prostate carcinoma cells in culture and in nude mice inhibits proliferation and tumorigenicity\(^{69}\). Therefore, like CDX2, NKX3.1 promotes differentiation and suppresses cellular proliferation, whereas its loss of function predisposes to cancer in a tissue-specific manner.

In humans, NKX3.1 is localized to a ‘hotspot’ on chromosome 8p\(^{68,71}\) that frequently undergoes allelic loss in PIN as well as in prostate carcinoma\(^{72,73}\). Similar to CDX2, NKX3.1 is genetically haploinsufficient, as the remaining allele is not mutated either in human cancer or mutant mouse models\(^{70,73}\). NKX3.1 undergoes epigenetic inactivation through the loss of NKX3.1 protein expression in PIN as well as prostate carcinoma\(^{69,72,70}\), and this loss of expression does not absolutely require genetic inactivation of one or more NKX3.1 allele\(^{69}\).

Several notable features of the loss of function of CDX2 and NKX3.1 in tumorigenesis might be of significance for other homeobox genes that are downregulated in cancer. First, CDX2 and NKX3.1 show tissue specificity with respect to their normal expression
patterns and the consequences of their loss of function in carcinoma. Second, their loss of function predisposes to, but is not sufficient for, carcinoma. Third, although these homeobox genes negatively regulate cell growth and tumorigenicity, their potency is relatively modest compared with ‘classic’ tumour-suppressor genes, such as TP53 (which encodes p53 in humans) and RB. Last, they are genetically haploinsufficient and undergo epigenetic inactivation through the loss of protein expression. These striking similarities could provide a general paradigm for studying the consequences of loss of function of other homeobox genes in cancer.

A model for homeobox genes in tumorigenesis

The consequences of deregulated homeobox genes for carcinogenesis can be interpreted as an extension of their normal function. Homeobox genes can be viewed as global regulators of growth and differentiation, with different classes of genes acting at different developmental stages and with specific members of these families being of particular relevance for specific tissue types. In normal tissues, their cumulative activities provide a balance between proliferation and differentiation, whereas perturbations of homeobox gene expression promote the transformed phenotype (Fig. 3). This view can account for homeobox genes that are negative regulators of differentiation and that have oncogenic activities. Members of this class include the HOX and MSX families, as well as those that are positive regulators of differentiation for which loss of function promotes transformation, such as members of the CDX and NKX families.

Homeobox genes are unlike ‘classic’ oncogenes or tumour-suppressor genes in several respects. First, their gain or loss of function does not seem to be sufficient for tumorigenesis; rather than being the driving force for carcinogenesis, their deregulation seems to tip the balance in one direction or another. Second, they show tissue-specific features that are not typically observed for ‘classic’ oncogenes or tumour suppressors, which frequently display broad specificities in many tumour types. Third, their deregulation in solid tumours might not involve mutations or altered functions, although this is not the case for translocations that involve homeobox genes in leukaemia (Box 1). Therefore, I propose that homeobox genes can be more precisely defined as positive or negative ‘tumour modulators’, rather than as oncogenes or tumour suppressors.

In conclusion, the evidence available supports the broad generalization that the gain and loss of homeobox genes promotes tumorigenesis as a consequence of their inappropriate effects on growth and differentiation. However, the current state of our knowledge is insufficient to establish a precise causal relationship between individual homeobox genes and the specific cancer phenotypes to which they contribute, and to understand how homeobox genes contribute to the tissue-specific features of cancer. Hints as to the nature of these relationships indicate that tissue specificity might reflect the differential activities of downstream target genes — as described for cyclin D1 as a downstream target of MSX genes, and BFGF as a target of HOX genes. So, future studies aimed at defining the selective functional outcomes of particular homeobox genes and specific tumour types might prove to be fruitful in understanding cancer phenotypes.