Abstract. Reasons for the lodgment of metastases from several types of solid cancer at apparently non-random sites have not been established. Recently, a group of genes expressed in human fibroblasts obtained from different anatomic locations was implicated in "positional" genomic information. Essentially, a Cartesian coordinate system identifying fibroblasts originally resident at anterior or more posterior, proximal or distal and dermal or non-dermal (heart, lung, etc.) locations was proposed. The determinants used for these identifications included HOX genes, central to embryonic segmental development, some of which are expressed in differentiated, post-embryonic cells. To the extent that HOX or other homeobox genes are expressed in ectodermal, mesodermal or endodermally-derived, malignantly transformed cells, they might contribute "positional" information to nidation of specific malignant clones at non-random sites. As understood in the past, interdiction of HOX or homeobox-related gene expression might reduce the probability of cancer cell implantation or alter their destinations in complex ways. Ideally, by interfering with HOX or other homeobox-related gene expression expression of antigenic determinants potentially contributing to their "homing" and nidation, reduced implantation of circulating cancer cells could render them more susceptible to systemic chemotherapy or immunotherapy, as demonstrated in mice. Furthermore, HOX or other homeobox genes or their products could provide novel intra- or extracellular targets for therapy.

Review

Does Homeobox-related "Positional" Genomic Information Contribute to Implantation of Metastatic Cancer Cells at Non-random Sites?

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Do HOX or Homeobox-related Genes Contribute Directly or Indirectly to Homing and Nidation of Metastatic Cancer Cells?

Two basic models have been proposed to account for the differential gene expression profile of fibroblasts located at different anatomical sites (1). In one model emphasizing the persistence of embryonic "positioning", considerable innate homology in profiles of cognate cells located at comparable anatomical sites is predicted. In the second model, local influences including epithelial-mesenchymal interactions are considered to determine possible similarity. Of course, blending of these models might occur.

Rinn et al. (2) have provided further evidence for the former hypothesis, in which the expression profile of fibroblasts at similar anatomical sites share features that identify the site from which they were harvested. Microarray gene expression profiles from 47 cultured human fibroblast samples, representing 43 different anatomical sites provided by 20 different donors were compared by a series of unsupervised and supervised hierarchical clustering studies. With successive refinements, a group of 337 genes that included augmented expression of functions associated with pattern and intercellular matrix formation, cell to cell signaling and that included a number of HOX genes, was identified. With this information, the original anatomical sites of the cultured fibroblasts could be distinguished as to anterior or posterior (caudal), proximal or distal and dermal or non-dermal (heart, lungs, prostate, intestines) location of the sampling.

In view of their importance in embryonic segmentation, the HOX genes were further studied. Forty-two homeodomain genes that included 12 HOX genes reflected the identification of fibroblasts according to their original location, suggesting a HOX "code" for positional identification. The genes expressed included HOXB2, B4, B5, B6, B7 and B9, limited to the trunk and non-dermal...
samples, while HOXD4 and D8 were expressed in trunk and proximal leg samples. HOXD4 was found by immunoblotting in fetal lung fibroblasts but not in fibroblasts from the foreskin. HOXA13 was expressed only in fibroblasts from distal sites, including the fingers, foreskin, feet and prostate. Confirming studies were performed with RT-PCR of several HOX genes and immunoblotting of HOXA13 from in vivo samples. Thus, fibroblasts from cognate sampling sites expressed greater transcriptional similarity than fibroblasts from anatomically distant sites. These results were considered consistent with a "positional" form of instruction of adult fibroblasts related to embryonic HOX gene expression and maintained by epigenetic mechanisms. Dorsal to ventral differences in fibroblast gene expression present during embryonic development were not found, implying that epithelial dominance dictated dorsal to ventral fates in the limbs.

Details of the anterio-posterior axis are complicated. Expression of the engrailed (en) gene in the posterior compartment mandates (P) identity; its absence yields (A) identity in the anterior regions (3). The en gene can repress or activate target genes. P cells expressing the EN protein are associated with hedgehog (hh) activation. To achieve A-P identities, en, a factor ci and hh are correctly expressed in the required compartments and finally the Polycomb group (PcG) genes maintain en repression in all embryonic segments, for example repressed hh in anterior segments.

Left-right asymmetry across apparent boundaries has been described for QL and QR neuronal cells in Chaenorhabditis elegans (4). QL neuroblast lineages migrated posteriorly, while QR neuroblast cells moved anteriorly, associated with HOX mab-5 gene expression in QL but not in QR cells, with an asymmetric response to a EGL-20/Wnt signal. A variety of mutants that disrupted these movements were described. A basis for bilateral, right versus left symmetric positioning in chick embryos depends upon sonic hedgehog activating the bone morphogenic antagonist, Cerebrus protein, with expression of nodal, activating the homeobox pitx2 gene, a member of the POU DNA-binding domain family, and repression of the snail gene, cSnR (5).

In QL cells, activin and its receptor are expressed, Fgf8 is activated, blocking expression of Cerebrus, allowing BMPO to inhibit nodal, snail gene activity and repression of pitx2. Similar events are considered to occur in human cells.

An additional concept is introduced in the HOX codes believed to underlie organ and cell-specific development of several organ systems, including the digestive tract (6), lung (7) and hematopoietic system (8). Sequential, unimpeded unfolding of HOX gene expression mandates the early normal growth and development of such complex organ systems, while interference with these processes results in various untoward disruptions of form and function. A further question is whether mammalian epithelial cells, malignantly transformed or not, are subject to "positional" controls comparable to those of fibroblasts. There is evidence that myogenic (9) and endothelial (10) cells exhibit some features of positioning, but additional evidence for "instructional" contributions, especially during early development between discrete epithelial-mesenchymal interactions contributing to localization and induction of specific cell types at different anatomic sites provides an alternate or additional explanation for these events (11).

Homeodomain and Homebox Gene-related Antigenic Determinants

In view of the many excellent reviews available (5, 12-21a,b), we will not attempt to review the topic in detail, but a few general comments could be useful. In mice and men, the HOX family includes 39 genes in 4 linkage groups (A through D) including some of the 13 homology (paralogue) groups, from nine to eleven members in each linkage group located on 4 different chromosomes, in order, A through D, numbers 7, 17, 12 and 2. Human paralogue groups 1-8 are related to the antennapedia (Antp) and groups 9-13 with abdominal-B (abd-B) of Home-C Drosophila genes. A larger group of non-HOX homeobox genes, of which HOX genes are a subset, are more variable and located at diverse loci. Class I HOX genes include 180 base pair homeobox DNA sequences, with a "signature" – ATTA – (and related -AT-) sequence(s), coding for 60 amino acid protein homeodomain containing sequences complementary to appropriate transcription promoters. HOX-related proteins originate from the 39 HOX genes while the more numerous "class II" homeobox-containing non-HOX genes give rise to additional proteins, such as the TALE (three amino acid loop extension) superclass, including homeodomain-related proteins from genes denoted as PBC, MEIS, IRO, TGIF. Class II homeobox genes give rise to additional homeodomain sequences and related proteins that have been denoted as POU, LIM, PAX, NK-2, Hix, TCL, NEC, emsZF, prd-like, prd, eve, lab, Dll, msh, NK-1, en, Abd-b Antp and the TALE superclass (18, 19, 21, 22). Individual classes include families of related genes such as Antp containing Dfd, Scol, Antp, Xbox and pd and TALE, mentioned above. This list is not in any sense meant to be complete and additional divergent homeobox genes and regions of micro RNA have been identified. The approximately 200 identified homeobox-containing genes give rise to some 1000 related proteins identified in various species (13). Reference (13) includes the following sites for listings of HOX-like genes (22a,b) and the National Human Genome Research Institute homeodomain resource database (23). The HOX-Pro database (22a,b) available at their web site provides several very informative diagrams depicting reported interactions as genetic clusters, networks and maps between a number of
HOX genes as these are affected by bone morphogenic proteins 2 and 4, Krox-20, Pbx-1, AP-2, Kraisler, retinoic acid via RAR and RXR and (probably) B-FGF.

Early expression of HOX and other homeobox genes are major determinants of embryonic segmental and antero-posterior organization of the basic body structure, preceded during embryogenesis by a sequence of events defined as maternal gene effects, expression of GAP genes and regulating pair-rule genes which divide the embryo into peri-axial segments, followed by segmental polarity expression in an antero-posterior axis within the segments which establish cell fates and followed by expression of homeotic "selector" and the downstream "realisator" gene expression (4 and Table I). The first three events are involved in activation of HOX and other homeotic genes leading to multiple functionalities mentioned above. "Selector" genes include HOX genes and "realisator" components populating the signal transduction pathways and implementing pathways. Homeobox transcription factors are implicated in cell division, adhesion, migration, forward and retrograde differentiation and programmed cell death, leading to component molecules contributing to these states.

These abbreviated comments, based on our understanding of the literature we have read, are intended only to suggest the evident complexity of a few of the HOX and homeobox interactions identified by the experts who actually study them, to suggest their potential contribution to "positional", possibly non-random genomic information or as subsequently transpired, even more generally to additional features of the "metastatic cascade" that might be co-opted during malignant transformation or subsequent metastasis.

**Primary and Metastatic Cancer Cells Modulate the Expression of Various HOX Genes**

A number of homeobox genes are expressed in differentiated normal (5-7, 9, 10) and in malignantly transformed cells (15-18, 21a,b; Table II). During embryological development, HOX gene expression occurs along an anterior to posterior, 3' to 5', spatial and temporal direction; this is not the case of expression between paralogue members (13). Presumably, post-embryonic expression of HOX genes is much less organized, more "individualistic". In mammalian cancers, HOX genes can be expressed or inhibited, compared to control cells; a few examples are presented in Table II. Five out of 39 HOX genes were expressed in normal esophagus, and 3 (HOXA7, A9 and C6) at significantly higher concentrations in squamous cell carcinomas (24). Eight other HOX genes (HOXA10, 13 B7, C4, C8, D9, D10, D13) were only expressed in the malignant cells. HOX C4, 5, 6 and 8 were overexpressed in malignant human prostate cell lines, primary tumor cells and lymph node metastases (25). Reduced expression (except for HOXC11) of numerous HOX genes was found in human invasive breast cancer (26). Overexpression of HOXA1 in mammary carcinomas increased cell anchorage-independent cell division, Bcl-2 expression, resistance to daunorubicin and caused neoplastic transformation (27).

HOXA5 expression induced apoptosis in MCF-7 cells that involved caspases 2 and 8 (28). Down-regulation of HOX, MEIS1 and MLL (mixed lineage leukemia) during normal hematopoiesis was absent in acute myelogenous leukemia cells and expression of HOXA9 and 10 and B3 and 4 was greatly reduced in cDNA from CD34+ compared with CD34+ normal cells (29). Chromosomal re-arrangements of HOXA9 or HOXD13 fused with NUP98, an activator of HOX gene expression, was present in some acute myeloid leukemia cells (30, 31) and gene expression profiling of myeloid leukemias identified 6 prognostic groups, one of which, cluster D, underexpressed HOXA9 and A10 (32). HOX gene expression in primary and metastatic cancers, cited below, can be similar or differ.

Many unresolved questions of interest include: What are the functions of HOX (and homeobox) genes that continue to be modulated in post-embryonic cells? How do these functions differ from their temporally and spatially integrated expression during embryological development? Is the concept of pleiotropy – different functions of a gene in different contexts – germane to understanding the consequences of post-natal expression and are any of these functions co-opted during malignant transformation, as seems to occur during leukemogenesis (30-32)?
Table II. Expression of HOX genes in some representative human cancers and derived cell lines compared to normal fibroblasts.

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>HOX Genes</th>
</tr>
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<tbody>
<tr>
<td>Leukemias:</td>
<td>HOXA9 or HOXD13 translocations with NUP98 (AML); MLL and HOX expression (ALL, AML); HOXA4 to HOXA11</td>
</tr>
<tr>
<td>Breast cancer:</td>
<td>+HOXA1, -HOXA10, +HOXB7, -HOX5; -HOX13;</td>
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<tr>
<td>Lung cancer:</td>
<td>+Wnt7A and HOX; HOXD3; +HOXA1, A5, A10, C5, D9, 10, 11 (squamous); +HOX5, A10 (adenocarcinoma); +HOXD3, SCLC, -HOXB and C loci, lower in metastatic ca; SCLC, +HOX7, A9 C 4, 5, 6, -HOXC5, D13; NSCLC, +HOX7, C5, D13, -HOXC4, C6</td>
</tr>
<tr>
<td>Esophageal cancer:</td>
<td>+HOXA10, 13, B7, C4, C8, D9, D10, D13</td>
</tr>
<tr>
<td>Colon cancer:</td>
<td>HOXB6, B8, HOXC9, various</td>
</tr>
<tr>
<td>Prostate:</td>
<td>+HOX C4, 5, 6 and 8; -HOX B13; -HOXD10</td>
</tr>
<tr>
<td>Renal cancer:</td>
<td>HOXA9; rarely +HOXD10, HOXC9; -HOXA2, E2, +HOXH3; group 10 (HOX1D, 2F, 3E, 4B differ from control); +HOXC11; -HOXA2, E2, +HOXH3</td>
</tr>
<tr>
<td>Bladder cancer:</td>
<td>+HOXC4, 5, 6, C11</td>
</tr>
<tr>
<td>Cervical cancer:</td>
<td>+(HOXA1, B2, B4, C5, C8, C10, D13 in 7 to 11 lines, absent in normal cells)</td>
</tr>
<tr>
<td>Ovarian cancer:</td>
<td>+HOXA9, 10, 11; +HOXB7; HOX7</td>
</tr>
<tr>
<td>Endometrial cancer:</td>
<td>-HOXA10</td>
</tr>
<tr>
<td>Melanomas:</td>
<td>HOXA1, 2, C4, B13 (metastases); HOXA11,13, B9, D12,13 &gt; naevus; HOXB7; -HOXD3; HOXC10,11,13 alter metastatic potential.</td>
</tr>
<tr>
<td>Thyroid:</td>
<td>HOXD9, not in ca</td>
</tr>
<tr>
<td>Astrocytes:</td>
<td>HOXA13, B13, D4, D9, D10, D13, differential expression, cell lines and cancers</td>
</tr>
</tbody>
</table>

"Control" expression of HOX genes in cultured fibroblasts (1, 2) related to position. HOXA10, 11, 13 proximal/distal upper forelimb, HOXA10 and 13, distal toe and foreskin; HOXB2, 4, 5, 6, 7, 9 (trunk and non-dermal); HOXC5, upper limb; HOXD4 and D8, trunk and proximal leg segments. Increased unless denoted as - or decreased expression. These representative examples are in no way exhaustive and most are cited in the references provided. Expression of HOXA10, 11, B2, 4, 5, 6, 7, 9 in SCLC, -HOXC5, D13; NSCLC, +HOXA7, C5, D13; -HOXC4, C6.

Arguments Restated

The Epithelial-Mesenchymal Transition and the Argument Restated

The epithelial-mesenchymal transition (EMT) is considered to be fundamental to embryogenesis and some of its properties are exhibited in the behavior of metastatic cancer cells. These include changes in cell morphology, increased resistance to programmed cell death as exemplified by apoptosis, reduced cell to cell adhesion with an ability to migrate, functional capabilities associated with homeobox, including HOX gene expression, which conceivably could be co-opted in some manner and contribute to the "metastatic cascade", including elements responsible for cancer cell "homing", among other properties (5, 11, 33, 34). In a number of experimental systems, these characteristics have been associated with increased expression of N rather than E-cadherin, of vimentin, ECM proteins, focal contact proteins, different integrins, nuclear localization of B-catenin and increased synthesis of transcription factors including Snail 1 and 2, Trist, E 47 among others that inhibit formation of E-cadherin. Malignant prostate cancers undergo a transition from an E-cadherin to the mesenchymal M form, exhibited a more aggressive clinical behavior (35). HOXB7 and HOXA10 have been implicated in the epithelial to mesenchymal transition of human breast (36) and uterine cancer cells (37), respectively. In the former, HOXB7 overexpression in primary (x3) and metastatic (x18) cancer cells was examined in transfected tissue culture lines, identifying increased bFGF synthesis, greater Ras and RhoA protein activity and phosphorylation of MAPK extracellular kinases, reversible by inhibitors, and overall implicating a HOXB7-induced bFGF – Ras / Rho pathway in EMT. Downregulation of HOXA10 in endometrial carcinoma correlated with less differentiation and increased methylation of its promotor. Increased expression of HOXA10 in cultured cells decreased their invasive properties; this was related to lesser expression of Snail, allowing increased expression of E-cadherin with increased cell adhesion (37).

In organisms capable of regeneration, such as salamanders, if an amputated limb is rotated 180 degrees and re-implanted, supernumerary digits in mirror-image symmetry are induced; an amputated limb is rotated 180 degrees and re-implanted, exhibiting a behavior (38). Proliferation and immune responses
characterized the former group and altered morphogenesis, glycolysis, including hypoxia-inducible factor and insulin-like growth factor-1 were present in the latter. The extent of overlapping functional categories (39) between the epithelial-mesenchymal transition (5, 11, 36, 37, 40), the wound healing-response (38) and established cancers, reflected in their respective gene profiles (e.g., 41) did not include prominent expression of HOX genes. In DNA microarray studies, epithelial cancer progression, employing mouse mammary EpH4 cells and several Ha-Ras-transformed variants, HOX8.1 (Msx2) and several agents able to affecting HOX gene expression during embryogenesis (BMP-4, KROX20 and 24 (EGR-2 and -1)), Kruppel-like factor (LKLF) and RAR-α) were represented in the epithelial to mesenchymal transition (40). Identified genes were grouped into 10 up-regulated and 9 down-regulated categories while some 15% of all regulated genes were found to be controlled at the translational level, as determined with polysome-bound mRNA expression profiling.

Signal transduction and implementing pathways detected in these and other studies of EMT, including wnts, Mets, src, sonic hedgehog, sox, PI3K/AKT, tyrosine kinases, RAS, EGF, HGF and TGF-β (42) might have been activated, at least in part by prior homeobox, including HOX gene transcription. In a study of CD34+ human cord blood cells transduced with HOXA9 or HOXA10, genes in the Wnt pathway including Wnt10B, Wnt receptors Frizzled 1 and 5, v-ets-related (ERG), Iroquois 3 (IRX3), aldehyde dehydrogenase-1 and a long chain acyl-CoA synthetase were up-regulated while HOXA10 repressed several heme and globin-related genes associated with differentiation; otherwise the effects on transcription of the two genes largely overlapped (43). Downstream modulation of stem cell genes related to energy metabolism, growth, division and differentiation by HOX genes situate them at points of fundamental cellular control. The concept of pleiotropy in which a gene may play a different role in different cells or in the same cell or cell lineage at different times may apply to both embryogenesis and to carcinogenesis and subsequent release, homing or nidation of transformed cells.

Metastases from several major epithelial cancers, including prostate or breast cancers to bone and instances of ipsilateral cancer metastases occur more often at characteristic, non-random sites than would be predicted by chance (34, 44-46). For prostate cancers metastasizing along the axial skeleton, the para-vertebral venous plexus has been considered contributory; alternately a clone of prostate cancer cells exhibiting expression of a co-opted or otherwise dysfunctional HOX (or homeobox)-related gene contributing during embryological development to anterior-posterior or dextral-sinistral or even superficial versus deep "positioning" could be suggested as contributory. For example, osteocalcin, whose expression is restricted to osteoblasts, which it activates, is regulated by homeodomain factors, AP-1 related proteins and other bone-restricted transcription factors (47). A paracrine or other mechanism of osteocalcin activation contributed to by activation of specific homeodomain genes active in metastatic cancer cells could in principle, contribute to induction of osteoblastic lesions. HOX9A9 represses TGF-β-induced osteopontin gene transcription via smads; smad 4 was a common signaling component for both BMP and the TGF-β pathways (48), activation of homeobox genes at the HOXc locus by induced interactions with growth factors, inflammatory cytokines and adhesion molecules has been reported (49), while bone extracellular matrix was reported to activate HOX genes, identified by multiple ATTA motifs in un-translated 3’ regions, in human LnCap prostate cancer cells cultured in the absence of androgens (50). LnCap cells studied in nude mice as LnCap/fetal fibroblast chimeric tumors that were androgen-independent all expressed HOX genes.

There is some disagreement concerning the importance of this transition in clinical cancer, based upon factors such as a lack of morphological differences between primary and secondary deposits, the presence in some metastases of E- rather than M-cadherin, and the lack of EMT in plant tumors (51). The presence of changes consistent with EMT at the invading front of metastases, numerous biochemical events associated with the EMT and the ability of metastatic cells to undergo a reverse differentiation from M- to E-like morphology and biochemistry, exhibiting reduced invasiveness seem to counter the objections (33).

Factors Affecting Dissemination and Nidation of Cancer Cells

To metastasize, malignantly transformed cells must successfully negotiate a metastatic cascade, encompassing local invasion from the primary, escape into the circulation, transport and arrest at distant sites, extravasation with local invasion and growth, including induction of angiogenesis (34). To abstract and grossly oversimplify, the majority of the relatively few migratory cancer cells that successfully implant initially require available routes for hematogenous or lymphatic spread (34, 52-54). During embryogenesis, the EMT transition is considered contributing to the migratory and invasive properties characteristic of mesenchymal cells (5) and of metastatic cancer cells. There is evidence that the ability of some malignant cells to colonize a distant site can depend upon unique properties of the migrating cells themselves, and less on the properties of the site. Human colon cancers exhibiting metastatic (M) or non-metastatic (N) properties in a host were transplanted to the colon or liver of nude mice (55). Both M and N cells invaded the colon; in contact with mouse liver, only M cells were invasive. This was considered dependent upon properties inherent in and unique to M cancer cells. Unidentified exchanges of metabolic or immunologic signaling between the M cell seed and mouse liver soil might have existed. Possibly N cells were unable to induce some hepatic
event necessary for their nidation or the liver lacked a component required for a potential N-cell invasion that was irrelevant to or provided for invasion by M cells.

Two major ideas underlying metastatic specificity entail either a homing explanation dependent upon the migratory cancer cell, the primacy of the soil relative to the cancer cell seed, or a blending of the two (56). In either case, cancer cells are thought to be engaged via a complementary interaction between adhesion receptors associated with/without secretion of chemotactic factors (57). An example of a second means of interaction is provided by the role of the stromal cell-derived CXCR4 pathway in prostate cancer metastatic to bone (58). In breast cancer metastases, chemokine receptors, such as CXCR4, CCX7 and their ligands on breast cancer cells, a parathyroid-related peptide (PTHrP) found in osteolytic lesions or endothelin-1 and platelet derived growth factor in osteoblastic lesions can be contributory (59). As reviewed in (60), HOX2A inhibits cartilage condensation and bone formation by inhibiting Sox9 expression and down-regulating Cdf1; HOXD11 and D13 reduce cartilage formation and length of the tibia and fibula; HOXA13 inhibits cartilage proliferation; HOXD and BMP-2 are concerned with anterior to posterior limb positioning and in the hindgut BMP-4 and Abd-B-like (paralog 9-13) HOX proteins function as downstream modulators of sonic hedgehog signaling. Bone morphogenic proteins can regulate HOX gene expression and the latter can regulate expression of the former (60). For example, Smad 1, responding to BMP, displaces HOXC8 from the promoter and activates osteopontin and osteoprogerin gene transcription. Smad 6 serves as a co-repressor of HOXC8. Additional details are provided in (60).

The developing concept of a pre-metastatic niche including locally increased fibronectin, increased bone marrow-derived cells expressing vascular endothelial growth factor receptor 1 and metalloproteinase activity at a site prior to the appearance of migratory cancer cells, in response to growth factors elaborated by the primary cancer (61, 62). Treatment of animal tumors with agents that disrupt the vascular system resulted in mobilization of bone marrow-derived circulating endothelial cells able to colonize the cancer, which anti-angiogenic drugs could at least partially inhibit (62).

Genes that inhibit cancer metastases without necessarily altering malignant transformation represent another potential signal mechanism that might be influenced by HOX or other homeobox gene modulation (63-65). For example, inhibition of Twist, an important regulator of morphogenesis and inducer of the EMT, with siRNA, reduced the number of tumor cells in the circulation of mice and subsequent metastatic lung cancers (66). High concentrations of twist in human lobular breast carcinomas correlated with invasiveness.

HOX-related Responses that Could Contribute Directly or Indirectly to Proximal or Distal Features of Cancer Homing and Nidation

Several descriptions of gene profiles associated with metastases from non-hematological cancers do not seem to include primary modulation of HOX genes (67a,b, except c to 70, to cite several, but see ref. 43; HOXA9 and A1O in normal cord blood cells; 31, 32 and leukemia and 67c, increased HOXA5 in post-chemotherapy versus primary chemosensitive ovarian cancers). The molecular signature of breast cancer metastases to bone marrow involved repression of genes associated with extracellular remodeling, adhesion, cytoskeletal changes and signal transduction, emphasizing RAS and Hif-1α, when compared with lymphatic metastases (67a). Comparison of normal and malignant colon resulted in identifying 584 genes whose expression between them differed (68). Major differences in genes concerned with proliferation, apoptosis and the immune response were emphasized, e.g. PI3K, ELF4eL3, CASP3, HLA-E and R2-m, in addition to numerous others. L’Esperance et al. found that 121 genes were often up-regulated and 54 genes down-regulated in post-chemotherapy-treated ovarian cancer samples, compared with the primary tumors (69). Up-regulated genes included a number concerned with chemoresistance, those down-regulated with chemosensitivity, proliferation, cell cycle control, tumor suppression and apoptosis. Genes associated with diverse metastatic human adenocarcinomas include metastasis suppressor genes Nm23, KiSS1, KA11, CAD 1, BRMS1 and MKK4 (70). Comparison of gene expression patterns from a variety of cancers identified 128 genes that could be refined to include 17, comprising 8 up-regulated and 9 reduced that distinguished primary and metastatic adenocarcinomas. An analysis of 13,000 genes in 11 breast and 11 colorectal cancers identified a number of cancer-related genes separated into nine categories such as cell adhesion and motility, signal transduction, transcriptional regulation (71). HOXA3, categorized as a regulator of transcription, was the only HOX gene included in this classification. A number of other cancer-related genes were denoted; conceivably some non-HOX homeobox genes or associated pathways or components related to them are represented.

It does appear that so far the most direct association between HOX gene expression and related properties is presented by normal hematopoietic development and acute leukemia. Lacking much information about the consequences of modulating (often inhibiting) HOX gene expression, with their largely unknown (in much detail) consequences of functioning as transcription factors, let alone possible effects of non-HOX homeobox genes, it is not surprising that identifying precise contributions to malignant cellular behavior in solid cancers, especially any with therapeutic applications, has been difficult. It may be that
the question is not whether HOX genes are demonstrably up-regulated, but rather that during post-embryonic malignant transformation, specific HOX genes are turned on but do not achieve the value selected to define significantly increased expression in microarray studies, turned off or even regulated post-transcriptionally, having initiated events that cannot be easily shown to have required HOX gene expression. Simple feedback or even reverberating circuitry is not difficult to model (15).

If the question is broadened to include the expression of non-HOX homeobox genes (e.g., 72, 73a,b), apparently not all of which function as transcription factors, a more robust argument might be developed. Genes activated by the homeodomain BARX2 protein studied in the MCF-7 breast cancer cell line by chromatin immuno-precipitation and other techniques identified 60 potential candidates that included transcription, receptor, ligand, cytoskeletal, CAM, signaling and enzyme activities, and others outside these categories (72). RNAi partially inhibited a number of genes related to growth and invasiveness of MCF-7 cells, and indicated coordination of several differentiation pathways. Some of the hundreds of genes identified (references 67-71, as representative examples) may have included components of pathways related to antecedent HOX (or homeobox) gene expression. An extensive survey of homeobox expression in cancer cells is complicated by variations in nomenclature, e.g. PDX-1 or pancreas-duodenum homeobox-1 has also been denoted insulin promoter factor-1, islet/duodenum homeobox-1, somatostatin transactivating factor-1, insulin upstream factor-1 and glucose sensitive factor (74a,b).

Factors affecting normal lympho-hematopoietic cell adhesion and homing, including participation of integrins, immunoglobulins, lectins, sialomucins, hyaladherin and CD 44, 38, 144 and a number of chemokines including CXCR1-5, CCR1-10, among others, have been identified (75). Inappropriate HOX and homeobox gene expression in cancer cells might contribute to particular mechanisms for metastatic homing; e.g. neural cell adhesion molecule promoter activity was enhanced by co-transfection with HOX -2.4 and 2.5 (76). Early contributions of HOX gene expression to the metastatic cascade may be necessary but themselves insufficient to mandate nidation.

Despite earlier interest in these matters, in general, studies of homeobox and HOX gene expression do not seem to have led to many potentially exploitable insights, even with the demonstration of differences in HOX gene expression between the primary and metastatic cells (77). However, more recent evidence of their participation in the development of pancreatic cancer implies a paradigm for contributions to malignant transformation in other cancers (78). Blocking sonic hedgehog with cyclopamine reduced the invasiveness of human pancreatic cancer cells implanted in nude mice, and combined with gemcitabine prevented metastases and reduced the size of the transplanted primaries (78). Sonic hedgehog increases ectopic expression of BMP-4 and specific HOXD gene expression, albeit in the chick (79), and has been implicated in early and later events underlying the development of pancreatic cancer (80). In the latter paper, increased expression of the transcription factor denoted as "gilll" caused a reduction in E-cadherin and an augmented EMT. While examples of a precise assignment of positional specificity in cancer cells due to HOX or homeobox-related transcription factors seem lacking, given the ancient and central role for many of these genes in organizing the mammalian body plan, and the possibility of their co-option during malignant transformation, considerable scope for oncological mischief seems implied. A few additional examples follow, the universality and implications of which are often not clear.

Earlier (proximal) events. During HL-60 monocyte differentiation, down-regulation of HOXA7 was necessary for cell adhesion and migration on fibronectin. HOXA7 is often up-regulated in acute myeloid leukemia, and its over-expression in HL-60 cells interfered with these interactions (81). Up-regulation of HOXA5 induced apoptosis in breast cancer cells that was mediated by caspases 2 and 8 (28); up-regulation of the HOXA1 homeobox gene induced increased proliferation, resistance to doxorubicin and malignantly transformed immortalized human mammary cells. Enforced overexpression of HOXAI1 promoted invasion in vitro and in vivo studies in nude mice. Over-expressed HOXB7 promoted the epithelial to mesenchymal transition of human breast cancer cells (36). Transcriptional repression of a limited area of the HOXA gene cluster in human breast cancer was due to aberrant DNA methylation (82) and associated with reduced expression of the HOXA gene cluster without evidence of consistent changes in paralogous HOXB,C and D clusters (26).

HOXB7 is underexpressed in some breast cancer metastases, while reduced HOXAI5 expression in some breast cancers is associated with limited p53 expression (83) and down-regulation due to methylation of HOXAl0 in endometrial cancers was associated with increased tumor grade (37). In primary and metastatic melanomas and 25 melanoma cell lines, HOXB7 constitutively activated fibroblast growth factor (83). Others noted expression of HOXAI, A2, C4 and B13 in distant melanoma metastases, without a relationship to their sites (84). HOXB7 was overexpressed in human ovarian carcinoma cells, and increased the intracellular accumulation of FGF, a ligand with angiogenic and mitogenic activities (86). The antigenic product of HOXB7 served as a growth factor for ovarian carcinoma cells, increasing the intracellular concentration of FGF. Down-regulation due to methylation of HOXAI0 in endometrial cancers was associated with increased tumor grade (37).
In a number of experimental systems, HOX genes can promote or retard angiogenesis, depending upon the circumstances (87). In addition to a number of homeobox-related genes, HOXD3 likely promotes invasive/migratory behavior and HOXB3 capillary morphogenesis of endothelial precursors as a response to angiogenic signals (88), HOXD10 suppressed angiogenesis of human microvascular cells (89), HOXA5 promoted while HOXD5 and D10 down-regulated suppressed angiogenesis of human microvascular cells (89), HOXD10 promoted while HOXD5 and D10 down-regulated angiogenesis, the former by suppressing pro-angiogenic genes including VEGFR2, ephrin A1, Hif1α and up-regulating thrombospondin-2 (90). Overexpressed HOXD3 induced a coordinate expression of metastasis-related genes with altered adhesion molecule and metalloproteinase activity, increased motility and invasiveness in human A549 lung cancer cells (91) that implicated TGF-β dependent and independent pathways (92), while transduction of antisense into human melanoma cells reduced these properties; control cells did not express HOXD3 (93). Expression of E-cadherin and plakoglobin declined, integrin alpha3 and beta3, matrix metalloproteinase-2 and urokinase-plasminogen increased, and integrin and N-cadherin were expressed. Blocking integrins with anti-integrins yielded increased haptotaxis to fibronectin. Homeobox D10 can phenotypically revert breast tumor cells (94). Overexpression of HOXC8 in prostate cancer was correlated with loss of differentiation (95). HOXB13-derived protein inhibits androgen-receptor-positive prostate cancer cells by interaction with the receptor itself (96). An extensive list of hormones including estradiol, progesterone, testosterone, vitamin D and especially retinoic acid via RAR and RXR receptors modulate HOX gene expression (97).

**Distal (nutation) events.** Examples in which HOX genes promote the EMT (36, 37) and modulate expression of specific integrins, cadherins, caveolins and other adhesion factors (98-100) provide the most direct mechanisms for potential homing to distant sites. Inhibition of caveolin in human breast cancers exhibiting suppressed HOXA expression (82) is a recent example. Caveolin, a protein component of caveolae, the non-clathrin coated invaginations of plasma membranes, inhibits anchorage-dependent growth and the invasiveness of human breast cancer cells (101). Evidence that some primary cancers can themselves evoke complementary responses at remote sites contributing to nidation further complicates this issue (61, 62). Homeobox A9 regulates transcription of the EphB4 receptor which affects endothelial cell movement and vessel formation (102), while the enhancer element in EphA2 is activated by HOXA1 and B1 proteins (103). HOX13 regulates the expression of bone morphogenetic proteins 2 and 7 by association with their enhancer regions, indicated by immunoprecipitation studies and partial correction of HOXA13 mutant phenotype after addition of BMP-2 or -7 (104). Bone morphogenetic protein-6, commonly present in prostate cancers, promoted osteoblastic metastases associated with induced SMAD phosphorylation of pre-osteoblast MC3T3 cells (105). Prostate-conditioned medium induced mineralization of these cells. Anti-BMP-6 medium reduced LuCap 23.1-induced osteoblastic activity of human fetal bone implanted in nude mice but left osteolytic activity unaffected. Intraosseous but not subcutaneous tumor sizes diminished. BMP-2, -4, -6 and -7 had no direct effect on prostate cell growth but BMP-2 and -6 increased their invasive activity; serum endothelin-1 levels did not differ among the groups studied.

**Several more specific biochemical mechanisms implicated in HOX gene expression.** A number of HOXC8 candidate target genes have been identified in mice overexpressing the gene (106). Based on oligonucleotide microarray data from C57BL/6J mouse embryo fibroblasts, 34 genes were altered at least 2-fold, 16 up- and 18 down-regulated. Expression of osteopontin was reduced and frizzled homolog 2 up-regulated, both about 4-fold and an interaction between the OPN promoter and HOXC8 demonstrated by chromatin immuno-precipitation. Overall, genes involved in apoptosis and in cell motility and proliferation were down-regulated, adhesion molecules mixed, increased expression of frizzled, a cell surface receptor for the WNT-B-catenin-TCF-signaling pathway important in several cancers, the potential utility of osteopontin as a marker for a number of cancers supported and findings generally consistent with an important role for HOXC8 expression in neoplastic development and further evolution were presented.

Targets of HOX genes in developing murine hindbrain and spinal cord, reviewed in (13), include discussions of cell adhesion molecules (N-CAM, L-CAM, cadherin 6, a-2-integrin, E-cadherin, aIb53, CD44, implicating HOXA1, HOXC8, HOXA9, the latter two inhibiting osteopontine transcription and themselves inhibited by Smads and their signaling in the TGF-β and bone morphogenetic pathway); the potential tumor suppressor mgl-1, a target of HOXC8; ephrin receptors, an IgCAM subfamily of receptor tyrosine kinases (RTK) expressed in rhombomeric patterns coordinately expressed with HOXA1, HOXB1 and HOXA2 with another ephrin, HOXA5 and HOXB7 and the G-protein pathway via Purkinje cell-specific pcp-2 inhibited by engrailed-2 (EN-2) but activated by the HOX genes post-natally, HOXB4 and the GTPase Ras superfamily member, Rap1, antagonizing Ras signaling of the ERK/MAPK pathway; protease inhibitors including serpins and HOXB5; transcription factors involved in HOX transactivations such as Otx homeobox genes and HOXB1, B2, B3, the Iroquois gene encoding TALE homeodomain proteins, downstream from HOXB4. The extent to which these associations are unique to the nervous system or have some applicability to other organ systems has probably not been established.

As cited in (43), in human cord blood stem cells, HOX A9 and A10 regulate genes involved with cell proliferation, including ALDH1, Iroquois 3 (IRX3), ets-related gene (ERG), very long chain acyl CoA synthetase with influence
on lipid, energy and ion metabolism, alcohol dehydrogenase and retinal dehydrogenase and the wnts pathway with effects on B-catenin-TCF, c-jun-N-terminal kinase (JNK) and the Ca$^{2+}$ and cGMP pathways. Lastly, HOX 11 interacts with protein phosphatases PP2A and PP1, interrupting a G2/M checkpoint (107).

**Conclusion**

 Initially we wondered if HOX or homeobox-related gene expression influenced homing and nidation of non-hematopoietic, solid malignancies to non-random sites. As information accumulated, additional influences on the events ascribed to the metastatic cascade had to be included.

 Provisionally, there appears to be no common pattern to activated or inhibited HOX gene expression, no simple identity with those reported present in fibroblasts (1, 2) or any proven relation with non-random metastases; at least we did not encounter such reports from our albeit limited search (Table II). To expect to identify such a simple correlation would probably have been surprising and actually unreasonable, given the limited information regarding downstream modulation of pathways by HOX or other homeobox gene-related transcription or the control of these genes and any directly or indirectly related proteins. Some cancers share modulated expression with normal fibroblasts of HOXA10, A11, HOXB2, 4, 6, 7, HOXC5 and HOXD4 and 8, activities which are increased or decreased compared with controls. In view of their potential functions, co-expression of several classes of HOX genes in normal fibroblasts and in examples of human cancers is of interest, but lacking sufficient knowledge of the consequences from modulated HOX and of homeobox gene expression, support for causal relationships between such comparisons and any consequences for cellular "positioning" remains suggestive but elusive.

 From their embryological antecedents, one might have anticipated metastases influenced by some HOX genes exhibiting "positional" information distributed in segmental patterns. Ipsilateral metastases in the absence of other affected sites is consistent with "positional" instructions related to anterior / posterior and dextral / sistral location, while dorsal / ventral positioning of cells occurs during the expression of various HOX codes (5-8). HOX genes at the 3' end can influence those in the preceding 5' paralogue, but generally posterior genes are said to be expressed to a greater extent than more anterior ones.

 The more numerous non-HOX homeobox genes do not seem to have been systematically surveyed for "positional" or other comparisons between fibroblasts and cancers. For example, overexpression non-HOX homeotic genes Cdx1 and Cdx2 promoted the malignancy of colon cancer cells, expressing a tumor-suppressor effect in the adult colon but a homeotic role during development (108). The mechanism of the suppressive effect and whether HOX or homeobox genes might contribute simply has not been determined. A sufficiently extensive analysis of even the available data from multiple sources might suggest linkages between homeobox gene expression and metastatic sites by particular cancer cell clones.

 Until the functions of several hundred homeobox genes, some 1,000 homeodomain proteins in various species and their interactions with the rest of their respective genomes are more fully understood, conclusions regarding putative effects of HOX and homeobox genes on the metastatic cascade, based on the available information and related inferences optimistically might support the Scottish legal verdict of "not proven".

 It seems surprising that the impact on cancer biology of homeotic genes, exerting such central effects on cell biology, has not been more profound. Considering the cyclic involvement of multiple signaling genes and their functional expression including FGF, notch, wnt and others mentioned above, during for example, mouse segmentation (109), the biochemical substrata for central effects on mammalian cell behavior, especially were such genes inappropriately activated or active ones inhibited in some disorganized manner, these and others should provide many potential sites for oncological mischief. Particular components of these networks and pathways – their "kernels", "plug-ins" and subroutines (15) – might be susceptible to co-option during carcinogenesis, and by their modulation confer properties on transforming cells associated with clinical malignancy, as has been understood in general terms for some time. Attempts directly to inhibit an overexpressed HOX gene might be partially thwarted by their functional redundancy, expression in normal cell growth and development or in initiating self-sustaining regulatory circuits no longer requiring their active participation.

 To the extent that modulation of specific cancer cell-related HOX and homeobox-related gene expression altered or even prevented homing and nidation of circulating cancer cells at random or non-random sites, a prolonged exposure to systemic immuno- or chemotherapy should be more lethal. The combination of gemcitabine and blockade of sonic hedgehog with cyclopamine in pancreatic cancer implanted in male athymic mice represents a new and innovative approach to treatment of cancer directed against a class of control molecules not frequently examined as potential therapeutic targets that can influence other regulatory factors such as bone morphogenetic proteins and HOX genes (76, 78-80, 93, 94). Increasing melanoma cell susceptibility to CTL-mediated lysis by increasing ICAM-1 expression (110), increasing antibody-dependent cellular killing with anti-E-cadherin antibody (111), or disrupting cell-cell interactions by inhibiting av integrins thus increasing antitumor and anti-angiogenesis activity in vivo have been reported (112). More recently, blockade of VEGF combined with GM-CSF tumor cell immunity prolonged survival of B16 melanoma and CT26 colon carcinoma cells in tumor-bearing mice (113).
A related benefit of interfering with homing/nidation concerns the cancer stem cells that presumably would not be able to implant. Interest is being focused on the role of cancer stem cells as the correct target for therapy of primary or metastatic cancer (114) and the development of cancer stem cells in hypoxic environments as major sources of therapeutic resistance (115). For almost all solid cancers, cancer stem cells in locations shielded from therapeutic agents, especially in regions of hypoxia, are thought to circumvent systemic therapies. Inhibiting nidation should reduce the number of stem cells in hypoxic or other protected regions of a metastasis. Release of non-replicative, more differentiated cells from a primary cancer that successfully lodge at some site and dedifferentiate to gain the ability to replicate seems less likely.

Accumulation of a more complete repertoire of HOX and homeobox transcription factor response and the pathways they modulate will likely provide important insights into the interpenetration of developmental processes as some of them may reappear in the biology of cancer cells.

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References

References for which we could only obtain abstracts are indicated with an asterisk.

Anderson et al: Homeobox-related “Positional” Genomic Information and Metastasis (Review)


employed, whether any causal relationship can be inferred from the study. For our purposes, those of us lacking direct experience in these several fields, some results are established through a species, experimental systems and normal or malignant cells. Many different ideas can be developed, based on data resulting from an extensive chain of observations. Arguments consistent with this interpretation in which almost any elements of a hypothesis may be considered to be in agreement, many of these "elements" may not maintain the "connectivity" of specific datum required for an extended and continuously valid argument, although some of the intermediate "steps" might be dispensable and not invalidate the overall contention.


Note in press: In a study in Nature (April 12, 2007), Massague and colleagues identified 4 genes, coding for proteins epiregulin, COX2, and matrix metalloproteinases 1 and 2 which when silenced individually, limited the growth of human breast cancers metastatic to the lungs in mice. Reducing the function of all four individually, limited the growth of human breast cancers metastatic to the lungs in mice. Reducing the function of all four simultaneously almost completely eliminated tumor growth by limiting their blood supply. In an article in Proc Natl Acad Sci USA on line (April 9, 2007), Massague and colleagues examined 18 genes denoted as constituting a “lung metastasis gene expression signature”, the LMS designation. Breast cancers among 738 analyzed exhibiting an active LMS were associated with metastases to lungs and with active vascularization.