LIM homeobox transcription factors, a novel subfamily which plays an important role in cancer (Review)

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Abstract. LIM homeobox genes are one of the most important subfamilies of homeobox genes which encode LIM-homeodomain (LIM-HD) proteins featuring two LIM domains in their amino termini and a centrally located HD that is used to interact with specific DNA elements in target genes. Numerous studies have reported their fundamental roles in the development of various organisms; however, little is known about their functions in cancer. Recently, research has shown that LIM homeobox genes also play an important role in cancer development. Among 12 human LIM homeobox genes, 10 LIM-HD proteins have been reported to be associated with cancer. In the present review, we mainly summarize the functions of these genes in various types of cancer and their potential as biomarkers and the related challenges. More in-depth research concerning LIM homeobox genes in cancer from a signaling pathway perspective may help to understand tumor profiles, establish biomarkers and guide choices for combinatorial drug therapies.

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1. Introduction

LIM homeobox genes are one of the most important subfamilies of homeobox genes. They encode a series of LIM-homeodomain (LIM-HD) proteins featuring two LIM domains in their amino termini and a centrally located HD that is used to interact with specific DNA elements in target genes (Fig. 1). This special structure can set LIM-HD proteins apart from other transcription factors of homeodomain superfamilies such as paired homeobox (PAX) proteins, Pit-Oct-Unc (POU)-HD and HOX (homeobox) cluster proteins (1,2). Their LIM domain contains two tandemly-repeated, cysteine-rich, double-zinc finger motifs that can be recognized by a number of co-factors which can mediate LIM-HD functions. These LIM domain-interacting proteins include NLI/LDB/CLIM/CHIP, MRG1, SLB and RLIM, a ubiquitin protein ligase. What is more, by means of the LIM domain, LIM-HD proteins can interact with other transcription regulators; thus, they can participate in a wide array of developmental events (3-6).

Mammalian genomes such as those of the mouse, rat and human contain at least 12 LIM homeobox genes (Table I) that encode key regulators of developmental pathways. Studies with mouse models and human patients have shown that these genes are critical for the development of specialized cells in multiple tissue types including the nervous system, skeletal muscle, the heart, the kidneys and endocrine organs such as the pituitary gland and the pancreas (1).

Although many researchers have reported their fundamental roles in the development of various organisms, little is known concerning the functions of these LIM-HD proteins in cancer. Recently, studies have shown that LIM homeobox genes also play an important role in cancer. Among 12 human LIM homeobox genes, 10 LIM-HD proteins have been reported to be associated with various types of cancer (Table II). In this review, we mainly summarize the functions of human LIM-HD proteins in cancer development and their potential as biomarkers and the related challenges. In addition, we discuss the role of these LIM homeobox genes in cancer from a signaling pathway perspective.

2. Relationship of LIM homeobox genes and cancer

LHX1 and cancer. LHX1, also called LIM1, is required for head, kidney and female reproductive tract development in

Table I. Human LIM homeobox genes in the HUGO Gene Nomenclature Committee (HGNC) database.

HGNC name	Symbol	Synonym symbol(s)	Chromosomal location
LIM homeobox 1	LHX1	LIMK4, LIM-1, LIM1, MGC126723, MGC138141	17q12
LIM homeobox 2	LHX2	LH2, MGC138390	9q33.3
LIM homeobox 3	LHX3	DKFZp762A2013, LIM3, M2-LHX3, CPHD3	9q34.3
LIM homeobox 4	LHX4	Gsh-4, Gsh4	1q25.2
LIM homeobox protein 5	LHX5	MGC129689	12q24.31-q24.32
LIM homeobox protein 6	LHX6	LHX6.1, MGC119542, MGC119544, MGC119545	9q33.2
LIM homeobox 8	LHX8	Lhx7	1p31.1
LIM homeobox 9	LHX9	/	1q31.3
ISL LIM homeobox 1	ISL1	ISL-1, ISLET1	5q11.1
ISL LIM homeobox 2	ISL2	FLJ10160, ISL-2	15q24.3
LIM homeobox transcription factor 1, α	LMX1A	LMX1-1, LMX-1, LMX1	1q22-q23
LIM homeobox transcription factor 1, β	LMX1B	LMX1-2, NPS1, MGC142051, MGC138325	9q33.3

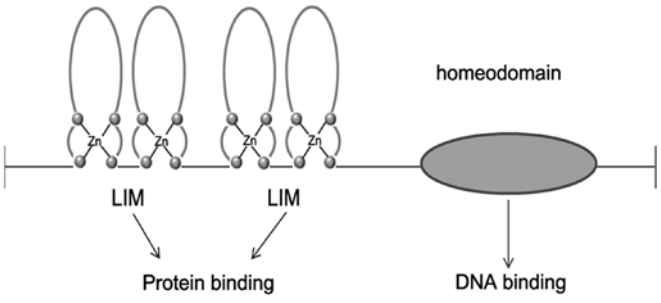

Figure 1. Schematic structure of LIM-HD proteins. The small gray spheres indicate the zinc-binding residues.

the murine embryo (7-9). Most LIM1 null mutants die with a headless phenotype around E10.5; the few surviving newborns had no kidney (7). Conditional ablation of LIM1 in the metanephric mesenchyme blocks the formation of nephrons at the nephric vesicle stage, leading to the production of small, non-functional kidneys that lack nephrons (10).

Guertl *et al* (11) investigated LIM1 expression during human renal development, in dysplastic kidneys and in renal neoplasms and found that LIM1 was detected in pretubular aggregates, S-shaped and comma-shaped bodies as well as immature glomeruli between 10 and 30 weeks of gestation. Eleven dysplastic kidneys showed no expression of LIM1. In contrast, 12 of 32 nephroblastomas showed nuclear positivity. One regressive nephroblastoma had diffuse expression of LIM1 in tubular structures, all others showed focal positivity in mesenchymal, blastemal and epithelial structures. Renal cell carcinomas revealed no expression of LIM1. Their study supports the concept of a causative role of LIM1 deficiency in the development of multicystic kidney. In a small subset of nephroblastomas with a more diffuse expression pattern, LIM1 might also contribute to the pathogenesis of these lesions.

Dormoy *et al* (12) further assessed whether LIM1 may be associated with tumorigenesis. They found that LIM1 is constitutively and exclusively reexpressed in tumors as a growth and survival factor in human clear cell renal cell carcinoma (CCC). More importantly, in nude mice bearing human CCC, LIM1 silencing abolished tumor growth through the same mechanism as *in vitro*. In LIM1-depleted cells and tumors, cell motility was substantially impaired due to the inhibition of expression of various proteins involved in metastatic spread, such as paxillin or tenascin-C.

LHX2 and cancer. The LHX2 gene functions as a transcriptional regulatory protein in the control of lymphoid and neural cell differentiation. LHX2 was initially identified as an early marker in B-lymphocyte differentiation (13). Overexpression of LHX2 in murine hematopoietic precursors leads to the development of chronic myeloproliferative disorders (14).

LHX2 was once found to be deregulated by juxtaposition with the IGH locus in a pediatric case of chronic myeloid leukemia (CML) in B-cell lymphoid blast crisis. The strong overexpression of LHX2 induced by t(9;14)(q33;q32) may play a recurrent role in leukemogenesis, specifically when it is in association with BCR-ABL chimera of the Philadelphia chromosome (Ph'), the hallmark of CML (15).

High levels of LHX2 expression were also observed in all cases of CML tested, regardless of disease status and may prove useful as a marker of CML for monitoring residual disease (16). Since LHX2 was mapped to chromosome 9q33-34.1, in the same region as the reciprocal translocation that creates the BCR-ABL. they also confirmed that the transcriptional activation of LHX2 in CML is likely due to a *cis*-acting effect, but not a *trans*-acting effect, of the Bcr-Abl fusion protein. The high level of LHX2 mRNA in CML cells probably can also be a consequence of the low level of methylation of the gene in leukemic cells (17).

Gorantla *et al* (18) reported the presence of uPA in the nucleus as well as the interaction of uPA with LHX2. LHX2 was overexpressed at the invasive front of tumors while completely absent in tumors downregulated for both uPAR and uPA. Suppression of the uPAR-uPA system retards angiogenesis, invasion, and *in vivo* tumor development in pancreatic cancer cells. Recently, LHX2 was also identified as a transcription factor functionally positioned downstream of p63 and NF-κB, but upstream of signals such as Wnt/β-catenin, BMP, and SHH that are required to drive activated stem cells toward terminal differentiation (19). This would indicate that LHX2 may behave like a gatekeeper molecule mediating the activation of cancer stem cells.

Notably, besides being overexpressed as an oncogene in cancer, LHX2 may be downregulated due to hypermethylation in cancer. Homeobox genes that are upregulated in cancer may be normally expressed during development and/or in undifferentiated cells, whereas others that are downregulated in cancer may be normally expressed in adulthood and/or in differentiated tissues (20).

Rahmatpanah *et al* (21) used an array-based technique called differential methylation hybridization (DMH) to study small B-cell lymphoma (SBCL) subtypes and found that hypermethylation of only one gene, LHX2, was present in all Non-Hodgkin's lymphoma (NHL) cell lines and a high proportion of patient samples.

Kim *et al* (22) utilized a genome-wide technique, methylated DNA isolation assay (MeDIA), in combination with high-resolution CpG microarray analysis to identify hypermethylated genes in breast cancer. LHX2 showed significantly higher frequencies of aberrant hypermethylation in primary tumors (43.6%; $P<0.05$) while frequencies were intermediate in paired adjacent normal tissues and absent in normal tissues. Moreover, a significant number of genes (2853; $P<0.05$) exhibited an expression-methylation correlation in breast cancer. Among these genes, LHX2 and LHX5 had prognostic value independent of subtypes and other clinical factors (23).

In lung cancer, Rauch *et al* (24) identified frequent methylation of homeodomain-containing genes including LHX2 and LHX4 using methylated-CpG island recovery assay (MIRA)-assisted microarray analysis. Although low levels of methylation were detected in some normal tissues removed by tumor surgery, methylation of LHX2 and LHX4 was more pronounced in tumor samples.

LHX3 and cancer. LHX3 null mice were found to have defective development of spinal cord motor neurons (25) and also to exhibit incomplete pituitary development (26,27). LHX3 functions to specify interneuron and motor neuron fates during development (28). To date, LHX3 has only been shown to be correlated with breast cancer. Dietrich *et al* (29) reported that elevated methylation of LHX3 was detected in invasive ductal carcinoma (IDC) and ductal carcinoma *in situ* (DCIS) compared with normal ducts, adenosis tissue, stroma and tumor infiltrating lymphocytes (TILs).

LHX4 and cancer. During embryonic and postnatal development, the LHX4 gene is expressed in hindbrain, cerebral cortex, pituitary gland and spinal cord, suggesting that LHX4 plays a role in nervous system development (30,31). Mutations in LHX4 genes are associated with combined hormone deficiency diseases in human and animal models (32,33).

Similar to LHX2, LHX4 can be upregulated by chromosomal translocations and downregulated by hypermethylation in cancer. Firstly, LHX4 mRNA was found to be expressed at high levels in the leukemic cells of patients and in an acute lymphoblastic leukemia (ALL) cell line due to the t(1;14)(q25;q32) suggesting a rare but recurrent genetic abnormality in leukemogenesis (34,35).

Moreover, de Bruijn *et al* (36) reported that the SS18 gene on chromosome 18 is fused to either one of the three closely related SSX genes on the X chromosome as a result of the synovial sarcoma-associated t(X;18) translocation. They found that endogenous LHX4 binds to the CGA promoter and that LHX4-mediated CGA activation is enhanced by the SS18-SSX protein, but not by the SSX protein and suggested that this novel protein-protein interaction may have direct consequences for the deregulation of SS18-SSX target gene LHX4 in the development of human synovial sarcomas.

Meanwhile, Goc *et al* (37) provided an initial report that simvastatin simultaneously modulated intrinsic and extrinsic pathways in the regulation of prostate cancer cell apoptosis *in vitro* and *in vivo* and also significantly reduced protein levels of pro-survival gene LHX4, but the exact function is yet to be determined.

In contrast, low expression of LHX4 was found in primary lung tumor samples (23) and hepatocellular carcinoma (HCC) (38) when compared with the expression level in paired non-cancerous tissues. Moreover, low expression of LHX4 was associated with tumor undifferentiation state and a high AFP level in HCC. Functional studies revealed that ectopic expression of LHX4 reduced AFP expression, which leads to the suppression of HCC growth. The results indicate that LHX4 functions as a potential tumor suppressor in hepatocarcinogenesis.

LHX5 and cancer. Human LHX5 is expressed in the adult central nervous system, including the spinal cord, thalamus and the cerebellum (39). The mouse LHX5 protein is closely related to the LHX1 LIM-HD factor, and complementary or overlapping roles of these two regulatory proteins have been suggested (40). Mice that are heterozygous for a LHX5 gene disruption appear normal, while most homozygous mice die a few days after birth (41).

Together with LHX2, LHX5 was found to have prognostic value independent of subtypes and other clinical factors in breast cancer (22).

LHX6 and cancer. LHX6 encodes an LIM-homeodomain protein involved in embryogenesis, more specifically in mammalian head development (42). Estécio *et al* (43) identified LHX6 as a new frequent cancer-associated hypermethylated CpG island in human head and neck squamous cell carcinoma (HNSCC). The hypermethylation of this fragment was detected in 13 of 14 (92.8%) HNSCC cell lines studied and in 21 of 32 (65.6%) primary tumors, whereas little or no methylation was noted in 10 normal oral mucosa samples. They extended this investigation to other cancer cell lines and methylation was found in those derived from colon, breast, leukaemia, lung and in 12/14 primary colon tumors.

Table II. Summary of studies on the role of LIM homeobox transcription factors in cancer.

LIM-HD	Main developmental function	Possible roles in cancer	Gene expression control mechanism	Related cancer	Refs.
LHX1	Essential for development of human renal and urogenital systems	Oncogene	Unknown	Nephroblastoma	(11)
				Clear cell renal cell carcinoma (CCC)	(12)
LHX2	Functions as a transcriptional regulatory protein in the control of lymphoid and neural cell differentiation	Oncogene	Overexpression due to chromosome translocation or hypomethylation	Chronic myeloid leukemia (CML)	(15-17)
			Unknown	Pancreatic cancer	(18)
		Methylation biomarker	Downregulated by hypermethylation	Non-Hodgkin's lymphoma (NHL)	(21)
				Breast cancer	(22,23)
				Lung cancer	(24)
LHX3	Required for pituitary development and motor neuron specification	Methylation biomarker	Downregulated by hypermethylation	Breast cancer	(29)
LHX4	Plays a role in nervous system development	Oncogene	Overexpression due to chromosome translocation	Acute lymphoblastic leukemia (ALL)	(34,35)
			Deregulated by protein - protein interaction	Synovial sarcoma	(36)
			Unknown	Prostate cancer	(37)
		Methylation biomarker	Hypermethylation	Lung cancer	(23)
				Hepatocellular carcinoma	(38)
LHX5	Essential role in brain development or compensatory actions of LHX1	Methylation biomarker	Hypermethylation	Breast cancer	(23)
LHX6	Involved in the control of differentiation and development of neural and lymphoid cells	Methylation biomarker	Hypermethylation	Head and neck squamous cell carcinoma (HNSCC)	(43)
				Cervical cancer	(44,45)
LHX7/8	No report				
LHX9	Plays a role in gonadal development and may be involved in the control of cell differentiation of several neural cell types	Tumor-suppressor gene	Downregulated by hypermethylation	Pediatric malignant astrocytomas	(47)
			Hypermethylation accompanied by transcriptional repression	Follicular lymphoma	(48)
SL1	Central to the development of pancreatic cell lineages and may also be required for motor neuron generation	Cancer biomarker	Unknown	Pancreatic neuroendocrine tumors	(50,54-57)
		Potential minimal residual disease (MRD) marker	Unknown	Neuroblastoma (NB)	(58)
ISL2	No report				

Table II. Continued.

LIM-HD	Main developmental function	Possible roles in cancer	Gene expression control mechanism	Related cancer	Refs.
LMX1A	Acts as a positive regulator of insulin gene transcription and it also plays a role in the development of dopamine producing neurons during embryogenesis	Oncogene	Unknown	Mucinous cystadenocarcinoma	(61)
				Glioma	(62)
				Pancreatic ductal adenocarcinomas	(63)
		Tumor suppression gene and methylation biomarker	Hypermethylation	Cervical cancer	(64-66)
				Ovarian cancer	(67,68)
				Bladder cancer	(69)
				Gastric cancer	(70)
				Colon cancer	(71)
LMX1B	Essential for the normal development of dorsal limb structures, the glomerular basement membrane, the anterior segment of the eye, and dopaminergic and serotonergic neurons	Regulate other oncogene overexpression	Through Wnt7a-LMX1b signaling pathway	Breast cancer	(74)

LHX6 methylation was also found in cervical cancer cell lines and cancer tissues (44). This epigenetic alteration in the LHX6 promoter begins at a relatively early stage as CIN I, suggesting its potential as a biomarker for early diagnosis (45). Moreover, overexpression of the LHX6 gene in cervical cancer cells was found to suppress the tumorigenic phenotype, as shown by soft agar colony formation and migration assays, suggesting that LHX6 could be a novel tumor-suppressor gene in the cervix.

LHX9 and cancer. The expression pattern and structural characteristics of LHX9 suggest that it encodes a transcription factor that might be involved in the control of cell differentiation of several neural cell types (46). The LHX9 gene is frequently silenced in pediatric malignant astrocytomas by hypermethylation and this epigenetic alteration is involved in glioma cell migration and invasiveness (47). LHX9 hypermethylation accompanied by transcriptional repression was found in follicular lymphoma (48).

ISL1 and cancer. The ISL1 transcription factor was initially cloned from pancreatic insulin-producing cells where it is able to bind the insulin gene enhancer (49). Mice deficient in ISL1 fail to form the dorsal exocrine pancreas, and islet cells fail to differentiate (50). In addition to its roles in the pancreas and heart, ISL1 is one of the earliest markers for motor neuron differentiation (51).

Thus, it is not surprising that ISL1 has been shown to be a sensitive marker of pancreatic islet cells and their neoplasms (50,52-56). ISL1 has been reported as a sensitive lineage-specific marker for pancreatic neuroendocrine neoplasms (NENs) and their metastases. Graham *et al* (54) also studied its specificity with large numbers of NENs from other parts of the gut or other organs. They found that ISL1 does not distinguish pancreatic NENs from duodenal and colorectal NENs, even when used in association with CDX2. On the other hand, in order to better understand the expression of the four transcription factors (TFs): ISL1, pancreatico-duodenal homeobox 1 gene product (PDX1), neurogenin 3 gene product (NGN3), and CDX-2 homeobox gene product (CDX2), that mainly govern the development and differentiation of the pancreas and duodenum, Hermann *et al* (57) studied their expression in hormonally defined pancreatic neuroendocrine tumors (P-NETs) and duodenal neuroendocrine tumors (D-NETs). They found a correlation between TF expression patterns and certain hormonally defined P-NET and D-NET types, suggesting that most of the tumor types originate from embryologically determined precursor cells. However, the observed TF signatures cannot distinguish P-NETs from D-NETs.

In addition, Cheung *et al* (58) explored potential minimal residual disease (MRD) markers differentially expressed in neuroblastoma (NB) tumors over normal marrow/blood with genome-wide expression profiling and identified 8 top-ranking markers: CCND1, CRMP1, DDC, GABRB3, ISL1, KIF1A, PHOX2B and TACC2. They were abundantly expressed in stage 4 NB tumors (n=20) and had low to no detection in normal marrow/blood samples (n=20). Moreover, expression of CCND1, DDC, GABRB3, ISL1, KIF1A and PHOX2B in 116 marrows sampled after 2 treatment cycles

was highly prognostic of progression-free and overall survival (P<0.001).

LMX1A and cancer. The LMX1A gene maps to 1q22-q23 and was proven to be a critical regulator of cell-fate decisions using genetic fate mapping in wild-type and LMX1A$^{-/-}$ mice (59). LMX1A was also found to play a pivotal role in the differentiation of human embryonic stem cells into midbrain dopaminergic neurons (60).

Recent evidence has shown an important role of LMX1A in cancer. For example, Lin *et al* (61) reported that higher immunostaining scores and the percentage of cells stained for LMX1A in mucinous cystadenocarcinomas correlated with T stage, American Joint Committee on Cancer clinical stage, poorer tumor differentiation, and poorer survival rate. In addition, a higher intensity of immunoreactivity for LMX1A correlated with more advanced grade in WHO grade I-III gliomas, but not in WHO grade IV tumors (62). In addition, higher expression of LMX1A and OPN was found to be highly correlated with histologic grade and pathologic stage of pancreatic ductal adenocarcinomas (63).

In contrast, LMX1A was identified as a metastasis suppressor in cervical cancer (64-66). It was once reported that the methylation of LMX1A correlated with the recurrence and overall survival due to the mechanisms affecting epithelial-mesenchymal transition (EMT) and stem-like properties in ovarian cancer (67,68). The methylation of LMX1A was also found to be associated with bladder cancer recurrence (69). Dong *et al* (70) found that the expression of LMX1A was significantly decreased due to the hypermethylation in gastric cancer tissues compared with normal tissues. Restoration of LMX1A induced cell apoptosis and suppressed anchorage-independent growth, suggesting that LMX1A is a potential biomarker for gastric cancer. Moreover, LMX1A hypermethylation was reported in a colon cancer cell line (HCT-116), but was demethylated in DKO cells in which two major DNA methyltransferases, DNMT1 and DNMT3b, were genetically disrupted (71). However, its role in transformation has not yet been characterized.

LMX1B and cancer. Together with LMX1A, LMX1B is part of a related subfamily of LIM-HD genes. Using targeted disruption of the mouse LMX1B gene, multiple functions of this factor have been uncovered. Mice lacking functional LMX1B exhibit numerous abnormalities, including a lack of ciliary body, iris stroma and corneal dysplasia (72,73).

To date, LMX1B has only been suggested to be associated with breast cancer. Rieger *et al* (74) found that LBH was overexpressed in highly invasive ER-negative, basal subtype human breast cancers and suppressed the differentiation of HC11 mammary epithelial cells. In further support of a clinical association of LBH with Wnt/β-catenin signaling, additional meta-analysis showed that LBH overexpression also correlates with Wnt pathway gene expression in colon cancer, which is primarily driven by Wnt activating mutations. Wnt7a-LMX1B signaling may be an important repressive mechanism that blocks Wnt/β-catenin target gene expression, and consequently ventral differentiation, in dorsal limb ectoderm (75). Thus, LBH may act as a downstream effector of this signaling in both normal and neoplastic epithelial development, which is under the tight control of antagonistic non-canonical Wnt7a signaling (76).

3. Targeting the developmental pathways to explore the role of LIM homeobox genes in cancer

Although numerous LIM homeobox genes are known to be involved in cancer, the roles of these genes in cancer remain unclear. Few LIM homeobox gene signaling pathway studies in cancer are available. Among these, LMX1A and LIM1 have been the subjects of a certain degree of intensive study.

Lai *et al* (65) demonstrated that LMX1A may have a critical role in preventing cervical cancer invasion and metastasis by inhibiting different aspects of EMT. They found that besides inhibiting cervical cancer invasion, the restoration of LMX1A altered expression of epithelial and mesenchymal markers in two cervical cancer cell lines (HeLa-3rd and CaSki). Furthermore, by analyzing TGF-β signaling, they found that BMP4 and BMP6 were downregulated by LMX1A. BMPs have been confirmed to be important components of LMX1A-dependent roof plate signaling, in which BMP6 is dependent on LMX1A expression during spinal cord development in mice (77). Moreover, LMX1A was found to be a mediator of early BMP signaling, and its activation of early roof plate development is dependent on BMP4 signaling in chicks (78). These complex regulatory networks of different BMPs and LMX1A in diverse microenvironments and tissues during embryonic development may also hint to its roles in cancer biology.

Transcription factors such as SNAIL, SLUG, TWIST, ZEB and FOXC2 are important markers of EMT. There is much evidence to confirm that several oncogenic pathways, such as growth factors (GFs), Ras, SHH, TGF-β, Wnt and Notch, may induce EMT. LIM homeobox genes such as LMX1A may also be important intermediate transcription factors which can induce or regulate core transcription factors and co-operate with them in target regulation, eventually leading to downregulation of epithelial genes and upregulation of mesenchymal genes (Fig. 2).

Regarding LIM1, it was found to be a downstream effector of SHH-Gli signaling functions and its expression is regulated by Pax2, FGFs and Wnt factors, which were confirmed to be involved in human tumorigenesis (79). Recently, LIM1 was found to be a growth and survival factor in human clear cell renal cell carcinoma through the activation of multiple oncogenic pathways including phosphoinositide kinase-3/Akt, MAPK and NK-κB pathways (13). To elucidate additional mechanisms accounting for the effect of LIM1 on tumor cell growth, western blot analysis and Proteome Profiler arrays (coated with apoptotic, phosphoproteins or angiogenesis pathways/markers) were used. The results showed that the various signaling pathways and molecular factors involved in cell apoptosis, proliferation, movement, angiogenesis were regulated at the level of expression and/or activation directly or indirectly by LIM1, such as phosphorylated Akt, Stats, cytochrome *c*, Fas, p38, p53, FGF, PDGF and EGF receptors as well as markers of cell movement, MMP8 andMMP9. Taken together, we suggest that LIM1 has a vital role in the expression and/or activation of various oncogenic and angiogenic pathways in human CCC (Fig. 3).

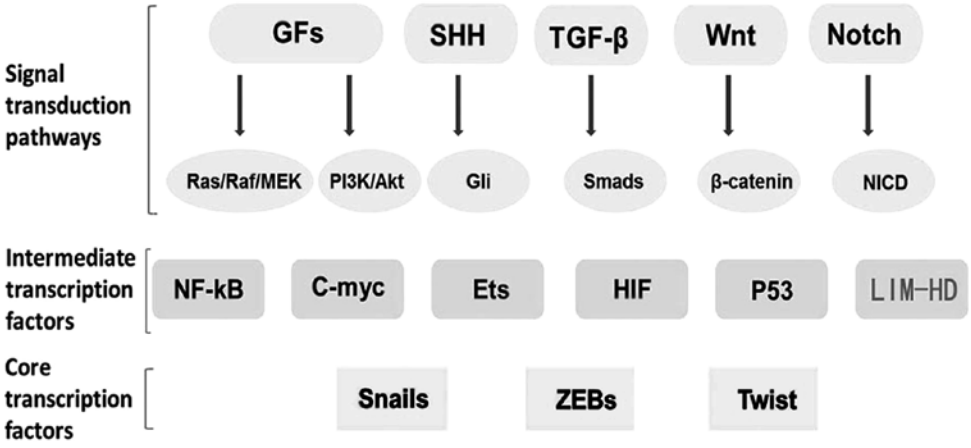

Figure 2. A conceptual layer overview of cancer EMT. LIM homeobox genes such as LMX1A may also be important intermediate transcription factors which can induce or regulate core transcription factors and co-operate with them in target regulation, eventually leading to downregulation of epithelial genes and upregulation of mesenchymal genes.

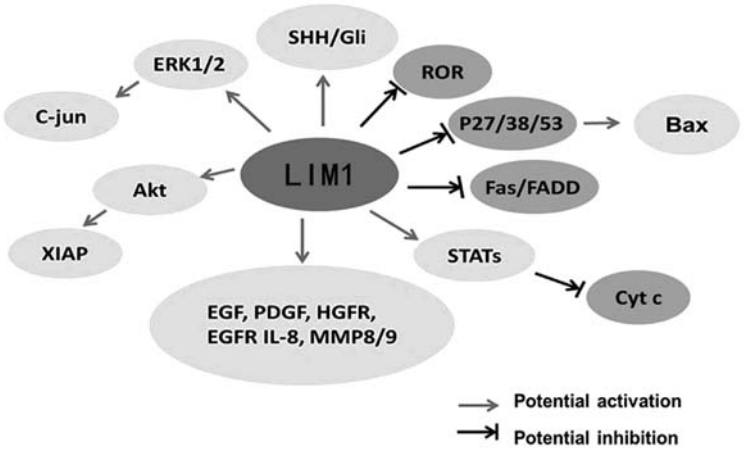

Figure 3. Potential LIM1 targets identified by Proteome Profiler array and western blot analysis in human clear cell renal cell carcinoma.

Since the developmental pathways involved in the genesis and the growth of an organ are also responsible for the development of a tumor, the idea that tumors 'hijack' for their own growth signaling pathways involved in normal development is emerging (80). The few available LIM homeobox gene signaling pathway studies in cancer reviewed above are consisted with this concept. Cancer often arises when normal cellular growth goes awry due to defects in critical signal transduction pathways; thus targeting these developemental pathways to explore the role of LIM homeobox genes in cancer will be effective.

In the present study, we document various confirmed signal pathway studies in development involving LIM homeobox transcription factors, which could provide various clues to explore the role of LIM homeobox genes in cancer.

Sato *et al* (81) reported that the kinase activity of WNKs is required for induction of LHX8 gene expression and the activation of SPAK/OSR1, and that the kinase-dead form of WNK acts as an actual dominant-negative form in the signaling pathway. Furthermore, the expression of LHX8 by either hypertonic or RA stimulation was required for the expression of both WNK1 and WNK4. Thus, this study provides initial evidence identifying the LHX8 gene acting downstream in the WNK-SPAK/OSR1 pathway, and demonstrates the significance of the WNK-OSR1-LHX8 pathway in neural development. WNK is a family of serine/threonine protein kinases that are characterized by a typical sequence variation within the conserved catalytic domain and could phosphorylate and activate SPAK or OSR1 kinases (82,83). The WNK-SPAK/OSR1 pathway is known to regulate various ion co-transporters and is widely conserved among many species (84,85). WNK1 is also required for cell division in cultured cells (86), and proliferation, migration and differentiation of neural progenitor cells (87). There is growing evidence for additional roles of WNK kinases in various signaling cascades related to cancer (88).

Wnt gain of function in cardiac progenitor cells was found to lead to expansion of ISL1-positive progenitors with a concomitant increase in FGF signaling through activation of a specific set of FGF ligands including FGF3, FGF10, FGF16 and FGF20. These data reveal that Wnt/β-catenin signaling promotes expansion of ISL1-positive progenitor cells through regulation of FGF signaling (89).

Roof plate (RP) development has been well studied in the spinal cord, where its specification relies on interactions between inductive signals of the TGF-β family produced by the adjacent epidermal ectoderm and intrinsic homeodomain

Table III. The methylation rate of LIM homeobox transcription factors in cancer cell lines and/or tumor tissues.

LIM-HD	Related cancer	Methylation rate	Refs.
LHX2	NHL	B-CLL/SLL, 46.6%; MCL, 41.6%; FL,73%	(21)
	Breast cancer	Tumor biopsies, 43.6%	(22,23)
	Lung tumor	Tumor biopsies, 58%	(24)
LHX3	Breast cancer	Unknown	(29)
LHX4	Lung tumor	Tumor biopsies, 75%	(23)
	Hepatocellular carcinoma	Unknown	(38)
LHX5	Breast cancer	Unknown	(23)
LHX6	HNSCC	Cell lines, 92.8%; tumor biopsies, 65.6%	(43)
	Colon cancer	Tumor biopsies, 85.7%	(43)
	Cervical cancer	Cell lines, 87.5%	(44,45)
LHX9	Pediatric malignant astrocytomas	High-grade gliomas, 55.6%;	(47)
		low-grade gliomas, 29%	(48)
	Follicular lymphoma	Unknown	
LMX1A	Cervical cancer	Tumor biopsies, 89.9%; cervical scrapings, 36%	(64-66)
	Ovarian cancer	Benign, 1.3%; borderline, 7.1%; malignancy, 34.9%	(67,68)
	Bladder cancer	Tumor biopsies, 9.43%	(69)
	Gastric cancer	Tumor biopsies, 82%	(70)
	Colon cancer	Carcinomas, 55%; adenomas, 42%; cell lines, 75%	(71)

NHL, non-Hodgkin's lymphoma; B-CLL, B-cell chronic lymphocytic leukemia; SLL, small lymphocytic lymphoma; MCL, mantle cell lymphoma; FL, follicular lymphoma; HNSCC, human neck squamous cell carcinoma.

transcription factors LMX1A and LMX1B (90-92), while similar mechanisms are involved in RP differentiation in the anterior midbrain. Alexandre *et al* (93) reported that the plasticity of the midbrain RP derives from two apparently antagonistic influences of FGF8. On the one hand, FGF8 widens beyond the neural folds the competence of the neuroepithelium to develop a RP by inducing the expression of LMX1B and Wnt1. On the other hand, FGF8 exerts a major destabilizing influence on RP maturation by controlling signaling by members of the TGF-β superfamily belonging to the BMP, GDF and activin subgroups.

A hallmark of cancer is reactivation/alteration of pathways that control cellular differentiation during developmental processes. Aberrant activation of developmental pathways such as Wnt, Hedgehog and Notch contributes to cancer development and progression, and these pathways are even intertwined at the molecular level. Thus, valuable prognostic biomarkers and the innovative therapies for cancer may be identified through these studies.

4. The potential of LIM homeobox genes as cancer biomarkers and the challenges

It is known that the genes that play important roles in the various steps of development may later be overexpressed or downregulated contributing to carcinogenesis. As reviewed above, several groups have reported the use of LIM homeobox genes as diagnostic and prognostic biomarkers, as certain gene expression profiles can be linked to tissue specificity, associated with early stages of carcinogenesis, and even linked to therapy-resistant disease resulting in a worse prognosis.

Analysis of the LIM homeobox gene abnormalities in cancer has demonstrated the presence of overexpression, at times, due to chromosomal translocations. However, more LIM homeobox genes are frequently downregulated in cancer due to hypermethylation (Table III), but not deletion or mutation.

Aberrant hypermethylation of the promoter regions of specific genes is a key event in the formation and progression of cancer and can be applied to cancer diagnostics in three ways: as a marker to detect cancer cells or cancer-derived DNA; as a marker to predict prognosis; and as a biomarker for the assessment of therapeutic response. Methylation analysis has an advantage in that it can be performed using chemically stable DNA (compared to RNA). Moreover, detecting gene methylation is easier than detecting gene mutation since the exact location of a mutation is usually unknown, making it difficult to specifically amplify DNA molecules with an embedded mutation in excess of wild-type molecules. More interestingly, detection of aberrant methylation can provide confirmation of the presence of intact cancer cells or cancer-derived DNA in bodily fluids, such as blood, urine, sputum, saliva and stool. Thus, their potential as biomarkers is growing.

Although numerous studies have demonstrated that LIM homeobox genes may serve as cancer biomarkers for diagnosis and prognosis, there are still many factors limiting the clinical application of these biomarkers. First, the majority of these studies were almost low level clinical reports, and lack of large sample, multi-center clinical studies exists. In order to confirm their clinical utility, enlarged sample size, more prospective studies, detection of the expression of methylated LIM homeobox genes in various periods of tumorigenesis and

testing using an independent set of collected patient samples must be carried out. Second, the mechanisms involving LIM homeobox genes and cancer are not totally understood, thus more in-depth study is required. Third, the technology analyzing gene methylation is not mature enough. Quantitative analysis of tumor sample is still a challenge. In the future, improving research methods and techniques is necessary. Fourth, as biomarkers, further detailed investigation is required to determine which markers have high sensitivity and specificity. Moreover, because of tumor heterogeneity, no single marker will likely be adequate. The inclusion of more new markers in a panel of hypermethylated genes in cancer potentially increases the sensitivity and specificity of tumor detection. Finally LIM homeobox gene immunohistochemical assessment or methylation detection should be combined with clinical and radiologic information to arrive at a definitive diagnosis.

5. Conclusions

LIM homeobox genes are one of the most important subfamilies of homeobox genes. As reviewed above, they not only participate in a wide array of developmental events, but also act as tumor-suppressor genes or oncogenes and are involved in various signaling pathways. Moreover, the silencing of LIM homeobox genes caused by hypermethylation is highly correlated with cancer. Yet, the mechanisms involved in the inhibition or promotion of tumorigenesis by LIM homeobox genes require further in-depth research. It has been reported that LIM homeobox genes may be used as cancer biomarkers for early diagnosis and prognostic evaluation. However, due to the complexity of clinical tumor samples, sensitivity and specificity of LIM homeobox genes have become the main challenges which hinder their clinical application. We believe that more in-depth research concerning LIM-homeobox genes in cancer from a signaling network perspective will be highly valuable in helping to understand tumor profiles, establish biomarkers, and guide choices for combinatorial drug therapies.

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