Cancer-Testis Antigen Lactate Dehydrogenase C4 in Human Cancers: Old and New Perspectives

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ABSTRACT

Mammalian mature testis and spermatozoa possess a unique lactate dehydrogenase (LDH) isoenzyme designated lactate dehydrogenase C4 (LDH-C4). While its existence only in mature testis and spermatozoa has already been attested, the latest evidence, including our study series, provides insights into LDH-C4 serving as cancer/testis antigen (CTA) in dozens of human cancers with ubiquitous aberrant transcription. We summarized recent findings on LDH-C4 to elaborate on its roles in human malignancies.

Keywords: Cancer/Testis Antigen; Lactate Dehydrogenase C; Cancer

Abbreviations: LDH: Lactate Dehydrogenase; CTA: Cancer/Testis Antigen; BC: Breast Cancer; HCC: Hepatocellular Carcinoma; RCC: Renal Cell Carcinoma; HNSCC: Head and Neck Squamous Carcinoma

Introduction

Lactate dehydrogenase (LDH) isozymes are abundant in various mammalian tissue types and accountable for the interconversion of pyruvate and lactate during the last step of anaerobic glycolysis [1-3]. Human LDH consists of four subunits of two types, A and B, linking together to form five tetrameric isoenzymes (LDH-1 to LDH-5). Following these findings, the sixth isoenzyme, designated as LDH-X, was detectable in the mature human testis in the 1960s [4,5]. LDH-X is also termed LDH-C4/LDHC as it is a tetrameric protein composed of four identical C-subunits. It, therefore, exhibits utterly distinct enzymatic, physicochemical, and immunological properties from the other isoenzymes [6-10]. Intriguingly, the current progress of LDH-C4 in human and murine reveals that though sharing high amino acid identities, human and mouse LDH-C4 are biochemically different from each other regarding some properties, particularly one that LDH-C4 is confined to mature testis, spermatocytes, spermatids, and spermatozoa [4-6, 11]. LDH-C4 is associated with glucose metabolism and is essential in the maintenance of ATP concentrations in spermatozoa [11,12-15].

Cancer/testis antigen (CTA) is, by definition, expressed only in the testis and tumor cells, absent in non-testis or noncancerous somatic cells [16]. LDH-C4 has been identified as a CTA molecule expressed in multiple malignancies, such as breast cancer (BC), lung cancer, and melanoma, with a high frequency of 47% in lung cancer, 44% in melanoma, 35% in breast carcinoma, and 15% in colon cancer. As suggested by an underlying mechanism, *LDHC* escapes from transcriptional repression, giving rise to significantly increased expression levels in a wide range of human tumor entities [17]. Our study series ascertained LDHC/LDH-C4 expressions in BC [18,19], liver cancer [20], nasopharyngeal carcinoma, lung cancer, and osteosarcoma and its predictive power as a biomarker [21]. Based on the latest discovery and our previous findings, emphasis will be given to the roles of LDH-C4 in human tumors.

LDH-C4 and Breast Cancer

Our BC research using an LDH-C4 inhibitor N-propyl oxamate has proven that endogenous LDH-C4 inhibition is crucial in the

exuberant invasion and metastasis of MDA-MB-231 BC cells [18]. However, this effect did not occur in LDH-C4-negative MCF-7 BC cells. Koslowski, et al. have found that the positive rate of *LDHC* in BC tissues is 35% [17]. But our results demonstrated much higher positive rates of serum and exosomal *LDHC* mRNA expressions of 91.66% (22/24) and 87.50% (21/24) in BC patients, respectively, versus the rates as low as 9.16% (11/120) and 6.67% (8/120) in healthy controls; the average LDH-C4 expression in the serum and exosomes of newly diagnosed BC cases were 9.79 and 8.87 folds of healthy controls [19]. Moreover, as the AUC values of serum and exosomal *LDHC* expression in discriminating BC from noncancerous specimens were 0.9587 and 0.9464, it could be used as a strong index for BC diagnosis [19].

Serum and exosomal *LDHC* levels are also associated with tumor size and HER2 and Ki-67 expressions in BC and with treatment efficacy and relapse, featuring a remarkable reduction in *LDHC* expression levels after the treatment or rebound *LDHC* levels since relapse [19]. By using high-throughput tissue microarray combined with the immunohistochemical assay, our finding demonstrated LDH-C4 protein expressions in BC tissues and their correlation with clinicopathological characteristics and BC prognosis [19]. LDH-C4 protein expression was predominantly detectable in the cytoplasm of BC cells but sparsely expressed in some nuclei. The positive rate of LDH-C4 in BC tissues was 91.55%, including low LDH-C4 expression (-/+) in 33.10% of BC tissues and high expression (+/++) in 66.90% of BC tissues. About 89% of adjacent normal tissues were free of LDH-C4, leaving only 11% with low LDH-C4 expression (-/+) [19]. Together with the survival analysis results that patients with high LDH-C4 levels were at higher risk of a poor prognosis, LDH-C4 is a useful indicator for prognosis prediction in BC.

LDH-C4 and Lung Cancer

Grunwald, et al. detected the mRNA expression levels of 7 CTAs (including LDHC) in NSCLC tissues and cell lines, and the positive rate of *LDHC* expression reached 25% in NSCLC tissues and completely undetectable in normal pulmonary specimens [22]. Histopathological subtyping revealed that *LDHC* mRNA was expressed in a high proportion of adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. They recommended the paired biomarker analysis of *LDHC* and CG genes for greater diagnostic power in NSCLC and used *LDHC* as a new tool for monitoring immunotherapy [22]. A study by Yen, et al. connected *LDHC* expression to cigarette smoking habits in lung cancer patients, especially in male patients with pleural effusion; the property of the aberrant *LDHC* expression between tumor entities of lung cancer makes the gene sensitive in predicting early-onset types like smoking-related lung adenocarcinoma [23]. The upregulation of *LDHC* gene expression is such a critical risk factor of lung cancer that LDH-C4 can serve as a potential marker for its diagnosis [23].

Consistently, our study with tissue microarrays also revealed that a high positive rate of LDH-C4 expression in adenocarcinoma of the lung of above 80% (unpublished data). Based on risk stratification, Kaplan-Meier curves disclosed a poor prognosis in patients with LDH-C4 overexpression (unpublished data). Correspondingly, a newly published study also confirmed that *LDHC* expression was significantly correlated with a poor prognosis of the lung adenocarcinoma patients; moreover, *LDHC* could promote proliferation, migration, invasion, and EMT in lung adenocarcinoma cells by triggering the PI3K/Akt/GSK-3β pathway [24].

LDH-C4 and Renal Cell Carcinoma

A study by Hua, et al. ascertained the relationship between LDH-C4 and clinical staging in renal cell carcinoma (RCC) [25]. Increased LDH-C4 expression indicated 'ferocious' invasion and metastasis of RCC cells and short-term survival, putting RCC patients at high risk of poor prognosis. So LDH-C4 may involve in both carcinogenic and malignant transformation regarding this primary malignant type [25]. Li, et al. utilized LDH-C4 down-expression in CAKI-1 RCC cells by siRNA silencing and found lowly expressed LDH-C4 markedly reduced the number of cells passing through a Matrigel-coated filter membrane, confirming the invasive and migrating potentials of LDH-C4-positive RCC cells indirectly [26]. Other studies of screening proteins related to cancer cell invasion and metastasis reported that endogenous LDH-C4 enhanced matrix metalloproteinase-9 (MMP-9) expression and lactic acid production, increasing the invasion and migration of malignant RCC cells [21,27,28]. Also, tumor-associated fibroblasts induced by substantial lactic acid build-up may propel hyaluronic acid secretion, creating an environment for the invasion and migration of aggressive RCC cells as well as exuberant metastasis [21].

LDH-C4 and Nasopharyngeal Carcinoma (NPC)

LDHC mRNA expression was proved undetectable in patients with head and neck squamous carcinoma (HNSCC) based on the analysis of CTA expression data from 51 cases [29]. However, our unpublished data showed that LDH-C4 protein was abundant in NPC tissues, particularly in the cytoplasm of NPC cells. LDH-C4-positive specimens accounted for 88.4% (114/129) of NPC patients, and cases of low (-/+) and high LDH-C4 expression (+/++) possessed 49.6% (64/129) and 50.4% (65/129), respectively. The tissue-specific LDH-C4 expression is also associated with clinicopathological manifestations, such as clinical staging and cervical lymphatic metastasis (unpublished data). The upregulation of LDH-C4 expression also links to shorter survival and a poorer prognosis in NPC versus patients with a low LDH-C4 level (unpublished data). This effect may be attributed to the underlying mechanism of accelerated growth, colony formation, and migration of CNE2 cells (a poorly differentiated NPC cell line) mediated by the rise of LDH-C4 expression (unpublished data).

LDH-C4 and Other Malignant Tumors

LDHC gene is also differentially expressed in melanoma and prostatic cancer and noncancerous specimens, implying its role in the occurrence and development of the two cancer types [17]. A study reported *LDHC* expression inhibition in osteosarcoma and that its complete transcript and metabolic characteristics were conducive to the early detection and diagnosis of osteosarcoma with high accuracy [30]. Our data for *LDHC* gene expression in hepatocellular carcinoma (HCC) demonstrated that 68% and 60% LDHC-positive specimens were detectable in the serum and serum-derived exosomes, showing AUCs of serum and exosomal *LDHC* in differentiating HCC from noncancerous controls of 0.8382 and 0.9451, respectively [20]. Consistently, LDH-C4 protein overexpression was found sensitive in predicting a poor prognosis of HCC patients [20]. Some studies have disclosed the aberrant *LDHC* transcript amplified in spermatocytic seminomas, osteosarcoma, and some melanoma [30-32]. Generally, the differentially expressed *LDHC* gene is detectable across various cancer types, and the expression status as well as the biological functions were summarized in Table 1.

Table 1: LDHC/LDH-C4 expressions and functions in human cancer types based on published studies.

Cancers	LDHC/LDH-C4 Expressions and Functions	References
Melanoma, breast, colon, prostate, lung, renal, ovarian, thyroid, cervical cancers; melanoma and lung cancer cell lines	Frequencies of *LDHC* expression in cancers: melanoma (7/16), breast (7/20), colon (3/20), prostate (3/8), lung (8/17), renal (4/7), ovarian (3/7), thyroid (1/4), and cervical cancers (5/6); melanoma (5/8) and lung cancer cell lines (2/6). *LDHC* activation was mediated by neither gene promotor demethylation nor hypoxia	[17]
Melanoma cell lines (A375M and C81-61)	CpG island hypomethylation and transcription factor Sp1 and CREB were found to play a major role in *LDHC* transcription	[30]
Lung adenocarcinoma	*LDHC* served as a candidate oncogene in the carcinogenesis of smoking-related lung adenocarcinoma	[23]
Non-small cell lung cancer	25% cases were *LDHC*-positive; *LDHC* could not be activated by genomic hypomethylation; LDH-C4 is a prognostic factor; LDHC enhanced proliferation, migration, invasion, and EMT in lung adenocarcinoma cells by triggering the PI3K/Akt/GSK-3β pathway	[22,24]
Hepatocellular carcinoma	68% and 60% of serum and exosomal *LDHC*-positive cases; patients with high LDH-C4 expression (+ + / + + +) in HCC tissues accounted for 55.84%. LDHC/LDH-C4 as a useful biomarker for early diagnosis, efficacy evaluation, relapse monitoring, and prognosis prediction for HCC	[20]
Breast cancer	91.66% and 87.50% of serum and exosomal *LDHC*-positive cases and 91.55% of LDH-C4-positive patients. LDHC/LDH-C4 exhibited high discrimination power in diagnosis, efficacy evaluation, relapse monitoring, and prognosis prediction for BC	[18,19]
Spermatocytic seminomas	*LDHC* was specifically expressed in spermatocytic seminomas	[32]
Osteosarcoma	*LDHC* was differentially expressed in osteosarcoma and normal specimens	[30]

Conclusion and Perspectives

Despite the conventional understanding of LDH-C4 as a key isoenzyme essential for sperm motility, capacitation, and fertilization, its encoding gene *LDHC* is a CTA family member and a potential therapeutic target of immunotherapy against several cancer types. The aberrant *LDHC* transcription predominantly participates in malignant behaviors, serving as a sensitive marker in tumor onset, development, and progression. However, the current evidence for LDHC's roles in cancer patients is insufficient, and what we have yet to discover includes other diverse biofunctions of LDH-C4 across various cancer types in patients, which require large-sample clinical studies with multiple independent sources.

Acknowledgement

Competing Interests

The authors have declared that no competing interest exists.

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