Overexpression of ASPH protein predicts poor outcomes in retroperitoneal liposarcoma patients

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To the Editor: Liposarcoma is a common subtype of retroperitoneal sarcoma. Due to the complicated anatomical heterogeneity of retroperitoneal liposarcoma (RPLS), it compresses and invades adjacent important organs, and massive bleeding frequently occurs during surgery. Therefore, a combined visceral resection is required, which means that RPLS surgery has greater difficulty and a lower complete resection rate compared to surgeries for other solid tumors. Although in our previous studies we found that intraperitoneal thermoperfusion chemotherapy including cisplatin may improve the prognosis of patients with RPLS,[1] surgery is the most effective treatment for RPLS, and adjuvant therapies have shown almost ineffective. There is a paucity of research focusing specifically on molecular mechanisms underlying RPLS pathogenesis. Consequently, no diagnostic biomarkers, druggable targets, or prognostic factors are available for RPLS.

Aspartate-β-hydroxylase (aspartyl-β-hydroxylase or asparaginyl-β-hydroxylase [ASPH]) is a highly conserved deoxidizing enzyme.[2] *ASPH* is essential for embryonic development. Normally, *ASPH* is silenced during adulthood and only expressed in the placenta. Abnormal upregulation of *ASPH* is observed in many malignancies, such as hepatocellular carcinoma, lung cancer, colorectal cancer, breast cancer, and pancreatic cancer. The enzymatic activity of ASPH depends on its catalytic domain, which hydroxylates aspartic acid or asparagine residues in epidermal growth factor-like repeats of several proteins (such as Notch receptors and Notch ligands) in the presence of iron, mediating cellular motility and differentiation. Previously, the correlation between *ASPH* mRNA levels and insulin receptor substrates, growth factors, insulin growth factor (IGF) receptors, Notch, Jagged, and HES was analyzed. Activation of the IGF-1/2 signal leads to upregulation of *ASPH* and thus Notch. Biologically, *ASPH* partially promotes tumorigenesis through activating Notch and SRC signaling pathways. Notably, the oncogenic properties of *ASPH* rely on its β-hydroxylase activity, which enhances proliferation, migration, invasion, and metastasis.

The Notch signaling pathway is indicated to be highly activated in RPLS, and the growth of liposarcoma cells can be significantly blunted by knocking down the Notch.[3] Hence, we explored if *ASPH* is associated with the tumor characteristics and clinical outcome of RPLS. We performed immunohistochemistry (IHC) to assess the expression profile of *ASPH* and evaluated its potential as a prognostic marker of RPLS.

Our study was approved by the Ethics Committee of Peking University International Hospital (No. WA2020RW29). Informed consent for clinical information collection was provided by patients or their guardians. There were 179 patients with RPLS admitted to the Department of Retroperitoneal Tumor Surgery of Peking University International Hospital from December 1, 2014, to February 28, 2018. Among them, 24 cases had surgical residues, five cases died due to serious post-operative complications, and 12 cases had extremely rare subtypes. These 41 cases were excluded in subsequent analyses. Thus, 138 cases (71 males, 67 females) were eventually included in this study. All cases were confirmed by histopathological and IHC examination. The mean age was 54.6 ± 11.5 years. Histologically, 82 cases had well-differentiated (WD) liposarcoma and 56 had dedifferentiated (DD) liposarcoma. None of the patients received chemoradiotherapy or targeted therapy before surgery. The general information and Fédération Nationale des Centres de Lutte Contre Le Cancer (FNCLCC) scores of these patients were retrospectively collected from the electronic medical record system.

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Formalin-fixed paraffin-embedded tissue blocks from the archived tissue bank were assessed by IHC. In brief, 4-μm-thick specimens were sectioned, deparaffinized in xylene, and rehydrated, followed by antigen retrieval in sodium citrate. Then, slides were incubated with a primary monoclonal antibody against *ASPH* (1:4000 dilution, courtesy of Professor Jack R. Wands, Brown University, USA) overnight at 4°C. Afterward, a biotin-labeled goat anti-mouse secondary antibody (Beijing Zhong Shan Jin Qiao Biotechnology, Beijing, China) was added. Sections were then counterstained with hematoxylin, dehydrated, and mounted. IHC was evaluated and scored by three independent pathologists. Four high-power fields (×200) were randomly selected for each slide, and the number of positive cells in a single field was counted and scored according to staining distribution as follows: 0 (negative), 1 (0–25%), 2 (26–50%), or 3 (51–100%). The average score of all fields was calculated. An average score ≤ 0.5 was set as negative to low expression, and >0.5 as moderate to high expression.

All patients were followed up by telephone or as an outpatient. The follow-up period was from discharge to October 2019 or death. The median follow-up was 21.5 months (4–53 months). Among the 138 patients, 84 relapsed, 43 died (all due to tumor recurrence and metastasis), and 14 were out of contact. The follow-up rate was 89.8%. SPSS 19.0 (IBM, Armonk, NY, USA) was used to analyze the data: the chi-squared test was used for count data, the Kaplan–Meier method was used for survival analysis, and a Cox regression model was used for correlation analysis. Statistical significance was established at $P < 0.05$.

Our study suggested that *ASPH* was overexpressed in RPLS compared to adjacent normal tissue. The overall positive rate of ASPH staining was 90.6%. The positive rate (90.2% [74/82]) in WD RPLS was significantly lower than that in DD RPLS (91.1% [51/56], $P = 0.028$). There was no difference in ASPH staining between patients divided by age, sex, tumor number, *P53* expression, or murine double minute 2 (*MDM2*) expression ($P > 0.05$). Patients with worse pathology subtypes ($P = 0.028$) and higher FNCLCC grades ($P = 0.007$) had stronger ASPH staining than others. Kaplan–Meier analysis showed that the overall survival (OS) of patients with high *ASPH* expression was much worse than that of patients with low expression ($\chi^2 = 6.56$, $P = 0.010$). The relapse-free survival (RFS) of patients with high *ASPH* expression was also much worse than those with low *ASPH* expression ($\chi^2 = 7.17$, $P = 0.007$). In the Cox regression univariate model, *ASPH* expression and FNCLCC grade were identified as prognostic factors ($P < 0.05$, Table 1). In the multivariate model, FNCLCC grade and *ASPH* expression were independent risk factors of RFS. The recurrence risk was 1.84 times higher in patients with a higher *ASPH* expression level compared to those with lower expression (odds ratio = 1.84, 95% confidence interval: 1.12–3.04, $P = 0.017$) [Table 1].

Compared with WD RPLS, DD RPLS is more aggressive, with unfavorable histopathological and clinical characteristics such as invasion into adjacent tissues at an early stage and a higher FNCLCC grade. In this study, *ASPH* expression in DD RPLS was significantly higher than that in WD RPLS and was related to FNCLCC grade. Thus, *ASPH* may play a role in the progression of RPLS, especially in DD RPLS.

Importantly, *ASPH* was an independent risk factor for relapse of RPLS. Patients with higher *ASPH* expression were more likely to relapse. The effect of *ASPH* expression on OS is similar to that on RFS; however, PFS and OS start to differ around 46 months after surgery. Considering the complexity and heterogeneity of RPLS patients' preoperative status, this unexpected intersection could possibly be explained by complicacy after surgery. Some patients with RPLS may survive a relatively long time by receiving repeated or even multiple operations. Some patients opt out of further treatment after suffering from economic difficulties, fear, and post-operative complications. All these factors make OS a less objective indicator than RFS.

It has been reported that *ASPH* promotes proliferation, migration, and invasion in various cancer types, including hepatocellular carcinoma, breast carcinoma,[4] and pancreatic cancer. Thus, *ASPH* acts as an oncogene and is expected to enhance aggressive or malignant cellular behaviors, possibly leading to a poor prognosis in RPLS. This hypothesis is under evaluation by cytological experiments. In this study, IHC data provided supporting evidence for an oncogenic role of *ASPH* in RPLS.

Previous studies have demonstrated that clinicopathological factors such as pathological subtypes and tumor size are associated with prognosis. Some studies have suggested that S-100, Ki-67, MDM2, and P53 are associated with the prognosis of RPLS. However, with a much larger cohort, neither *MDM2* nor *P53* were found to be a prognostic factor. Instead, *ASPH* has been identified as a potential prognostic factor in RPLS. As the embryonic origins of RPLS are different from cancers that originate from epithelium, few tumor biomarkers are valid in RPLS. However, before a deeper examination of the molecular mechanisms of RPLS, a pan-cancer evaluation of the utility of *ASPH* as a biomarker would be the first choice in developing it as an RPLS biomarker.

The biological characteristics of RPLS are different from carcinomas. RPLS tends to grow expansively and is prone to post-operative recurrence, but rarely exhibits distant metastasis. Thus, RPLS patients receive repeated and multiple surgical treatments. It is extremely important for such patients to prolong OS and disease-free survival without recurrence after surgery, and to gain time and space within the body for the next surgical resection. Our results suggest that patients with high-*ASPH* RPLS should be considered at high risk of relapse and should receive a much closer observation and longer follow-up.

Inhibiting the expression or enzymatic activity of *ASPH* could undermine the proliferation, migration, invasion, and metastasis of various tumor cells. In addition, *ASPH* can serve as an immunotherapy target for liver cancer.[5] Further studies are needed to verify the effects of *ASPH* on RPLS tumor cells, which would help to determine if *ASPH*

Table 1: Univariate and multivariate Cox regression analysis of RFS in RPLS patients.

Characteristics	Univariate		Multivariate	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age	1.01 (0.99–1.03)	0.164	–	–
Sex, male *vs*. female	0.74 (0.48–1.16)	0.194	–	–
Multiple tumors, yes *vs*. no	1.14 (0.72–1.82)	0.580	–	–
FNCLCC stage		0.001		0.002
II *vs*. I	1.48 (0.89–2.46)		1.28 (0.76–2.16)	
III *vs*. I	2.57 (1.48–4.48)		2.41 (1.38–4.22)	
P53 staining, positive *vs*. negative	1.33 (0.83–2.10)	0.233	–	–
MDM2 staining, high *vs*. low	0.88 (0.47–1.66)	0.693	–	–
ASPH score, negative-low *vs*. moderate-high	1.89 (1.16–3.07)	0.010	1.84 (1.12–3.04)	0.017

ASPH: Aspartate-β-hydroxylase; CI: Confidence interval; FNCLCC: Fédération Nationale des Centres de Lutte Contre Le Cancer; MDM2: Murine double minute 2; OR: Odds ratio; RFS: Relapse-free survival; RPLS: Retroperitoneal liposarcoma.

can be used as a druggable target or an immunotherapeutic target for (neo)adjuvant treatment of RPLS.

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Conflicts of interest

None.

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