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Retrospective Study

High patatin like phospholipase domain containing 8 expression as a biomarker for poor prognosis of colorectal cancer

Zhou PY *et al*. PNPLA8 and colorectal cancer

Peng-Yang Zhou, De-Xiang Zhu, Yi-Jiao Chen, Qing-Yang Feng, Yi-Hao Mao, Ao-Bo Zhuang, Jian-Min Xu

Abstract

BACKGROUND

Patatin like phospholipase domain containing 8 (PNPLA8) has been shown to play a significant role in various cancer entities. Previous studies used to focusing on its antioxidant role and lipid peroxidation. However, the role of PNPLA8 in colorectal cancer (CRC) progression is unclarified.

AIM

To explored the prognostic effects of PNPLA8 expression in CRC.

METHODS

A retrospective cohort containing 751 consecutive CRC patients was enrolled. PNPLA8 expression in tumor samples was evaluated by immunohistochemistry staining, and semi-quantitated with immunoreactive scores. CRC patients were divided into high and low PNPLA8 expression groups based on the cut-off values, which were calculated by X-tile software. The prognostic value of PNPLA8 were identified using univariate and

multivariate cox regression analysis. The overall survival (OS) rates of CRC patients in the study cohort were compared with Kaplan-Meier analysis and Log-rank test.

RESULTS

PNPLA8 expression was significantly associated with distant metastases in our cohort (P = 0.048). CRC patients with a high PNPLA8 expression indicated poor OS (median OS = 35.3, P = 0.005). CRC patients with a higher PNPLA8 expression at either stage I and II or stage III and IV had stastically significant shorter OS. For patients with left-sided colon and rectal cancer, the survival curves of two PNPLA8-expression groups showed stastically significant differences. The multivariate analysis also confirmed that high PNPLA8 expression was a significantly independent prognostic factor for overall survival (hazard ratio HR = 1.328, 95%CI 1.016-1.734, P = 0.038).

CONCLUSION

PNPLA8 is a novel independent prognostic factor for CRC. These findings indicate PNPLA8 as a potential target in clinical CRC management.

Key Words: Biomarker; Colorectal cancer; Expression level; Overall survival; Patatin like phospholipase domain containing 8; Prognosis

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Core Tip: Patatin like phospholipase domain containing 8 (PNPLA8) has been shown to be associated with a variety of cancers, but its role in the progression of colorectal cancer (CRC) is unclear. In this study, 751 consecutive CRC patients were retrospectively analyzed. The results of this study indicate that PNPLA8 is a new independent prognostic factor for colorectal cancer. High expression of PNPLA8 in

colorectal cancer leads to impaired survival. These findings suggest that PNPLA8 is a potential target for clinical colorectal cancer therapy, providing important insights to help personalize therapy for patients with colorectal cancer.

INTRODUCTION

Colorectal cancer is among the deadliest tumors[1]. The only curative treatment for localized colorectal cancer is surgery and patients with lymph node metastases are usually advised to undergo adjuvant chemotherapy[2]. The relatively low 5-year survival rate of about 56.9% is further affected by inadequate screening methods and increasing resistance to chemotherapy during the clinical course[3,4]. Currently, several reliable prognostic factors are widely used in clinical practice, such as molecular subtype, therapeutic response of previous adjuvant chemotherapy, time between adjuvant therapy and metastasis development (shorter is associated with poorer prognosis), comorbidities, and frailty[5,6]. Therefore, considering the heterogeneity of colorectal cancer is essential for developing new prognostic and therapeutic strategies. However, the widely accepted new prognostic biomarkers are scarce.

Patatin-like phospholipase domain-containing protein (PNPLA8), also termed Ca^{2+}-independent phospholipase A2γ (iPLA2γ), is addressed to the mitochondrial matrix, where it may manifest its unique activity to cleave phospholipid side-chains from both *sn-1* and *sn-2* positions, consequently releasing either saturated or unsaturated fatty acids, including oxidized fatty acids[7]. As a calcium-independent and membrane-bound phospholipase, PNPLA8 catalyzes the esterolytic cleavage of fatty acids from glycerophospholipids to yield free fatty acids and lysophospholipids, hence regulating membrane physical properties and the release of lipid second messengers and growth factors[8,9]. It is essential for maintaining efficient bioenergetic mitochondrial function through tailoring mitochondrial membrane lipid metabolism and composition[9]. Mutations in *PNPLA8* gene have been identified to be linked with multiple diseases such as mitochondrial myopathy with lactic acidosis and mitochondrial myopathy[10]. Recently, it was found that the dysregulation of iPLA2γ can therefore be a critical factor

in the development of many diseases[11,12], including metabolic diseases and multiple types of cancer, such as colitis and colorectal cancer[13]. However, the expression status of PNPLA8 in colorectal cancer and its relationship with clinicopathological features and prognosis are largely unknown so far.

In this study, to investigate the potential biomarker value of PNPLA8, 751 cases of tumor samples from a cohort of CRC patients were selected to analyze PNPLA8 protein expression by immunohistochemical staining. Additionally, concenrtrated analyses on the correlations between PNPLA8 expression and overall survival of CRC patients in this cohort were conducted to unveil the prognostic significance of PNPLA8 in colorectal cancer. Our results suggest that a higher PNPLA8 expression might be an independent predictor for poor prognosis in patients with colorectal cancer, which could be potentially used to guide the clinical management of patients with CRC.

MATERIALS AND METHODS

Patients and specimens

A total of 751 patients with CRC that were admited to Zhongshan Hospital, Fudan University (Shanghai, China) between May, 2008 and November, 2012 were retrospectively enrolled in this study. The inclusion criteria were as follows: (1) receiving primary radical resection; (2) pathologically confirmed colorectal adenocarcinoma; (3) no treatment before surgery; and (4) clinicopathological data available. CRC patients with radical resections of synchronous liver metastases were also included. CRC cancer stages were defined according to the International Union Against Cancer/American Joint Committee on Cancer TNM classification 8th edition. The diagnostic procedures were concluded before the current study was conducted. During the analysis, the observers were fully blinded for patients' data. The median follow-up time of the patients cohort was 46.1 mo (IQR = 32.9-59.5).

This study was approved by the Clinical Research Ethics Committee of Zhongshan Hospital, Fudan University. Informed consent was acquired from all patients of the

primary cohort for the acquisition of clinical and pathological information and the use of surgical specimens.

Immunohistochemistry

Formalin-fixed paraffin-embedded surgical specimens were used for tissue microarray (TMA) construction and subsequent immunohistochemistry study as described previously[14]. Standard procedures were used to determine the levels of PNPLA8 expression in CRC tumor samples. After being dried overnight at 37 ℃ and deparaffinized in xylene, the TMA slide was rehydrated through graded alcohol and then immersed in 3% hydrogen peroxide to block endogenous peroxidase activity. After that, the sections were pretreated in a microwave oven (14 min in sodium citrate buffer, pH 6) and then incubated with 10% normal goat serum for 30 min. Primary antibody (rabbit anti-human PNPLA8 polyclonal antibody, ab223726, Abcam; diluted 1:150) was applied overnight in a moist chamber at 4 ℃. After the primary antibody was washed off with PBS, the secondary goat anti-rabbit antibody (ab6721, Abcam; diluted 1:10000) was applided. Reaction products were visualized by incubation with 3,3'-diaminobenzidine and then counterstained with hematoxylin. Negative controls were treated identically, but with the primary antibody omitted. In addition, the paracancerous tissue were used as controls.

Evaluation of immunohistochemical staining

Two independent pathologists who were blinded to the clinical data evaluated the immunostaining and the results were averaged. In case of significant discrepancies, a final score was established by reassessment on a double-headed microscope and a third person was asked to re-score the results and choose the value with the closest score. The scores for PNPLA8 intensity were set as follows: '+++' was 3; '++' was 2; '+' was 1; and '−' was 0. The area scores for PNPLA8 expression were set as follows: '1' (0%-25% positive cells among all tumor cells), '2'(25%-50% positive cells), '3' (51%-75% positive cells), and '4' (more than 75% positive cells). The final score for PNPLA8 expression was

the intensity score multiplied by the area score, resulting in a final score ranging from 0 to 12. Boundaries were based on the results from X-Tile Software (Yale University, version 3.6.1), and a final score of 0-8 was considered as low PNPLA8 expression while 9-12 as high PNPLA8 expression.

Statistical analysis

The statistical analysis was performed using the SPSS 23.0 (IBM, Armonk, NY, United States). The association between clinicopathological features and PNPLA8 expression were accessed by Chi-square test or Fisher's exact test as appropriate. Kaplan-Meier analysis and Log-rank test were performed to evaluate the relationship between PNPLA8 expression and overall survival (OS). Univariate cox regression analysis was performed to identify the independent prognostic factors among clinicopathological features and other information. Those factors with $P < 0.1$ in univariate cox regression analyses were included in the multivariate cox regression analysis. A two-sided $P < 0.05$ was considered statistically significant. To obtain the best prognostic efficacy, the cut-off values of PNPLA8 score were calculated using X-Tile Software (Yale University, version 3.6.1) based on the OS data[15].

RESULTS

Clinicopathologic characteristics of the enrolled patients with CRC

The clinicopathologic characteristics of the enrolled colorectal cancer patients are listed in Table 1. Approximately half of the patients (53.7%) were over 60 years old, and their ages ranged from 19 to 90 years with a median age of 62 (SD, 12.3) years. The male to female ratio was 60.3: 39.7. The patients with CEA value over 5 ng/mL accounted for 47.3% of total patients, while those with CA199 value more than 37 U/mL accounted for 19.2%. The tumor location was categorized as right-sided colon in 209 cases (27.8%), left-sided colon in 200 cases (26.6%), and rectum in 342 cases (45.6%). There were 323 cases (43%) with tumor size over 4.0 cm, while the majority of all the cases were non-mucinous in terms of primary histological type. For primary tumor differentiation, 497

cases (66.2%) had well/moderate differentiation, while other 254 cases (33.8%) were poor/anaplastic in tumor differentiation. TNM Staging results showed that a small portion of patients (197 cases, 26.2%) were at active metastasis stage, whereas only 96 cases (12.8%) and 56 cases (7.5%) showed vascular invasion and nerve invasion, respectively.

Correlations between PNPLA8 expression and clinicopathological parameters

We next exmined the expression of PNPLA8 in tumor samples using immunohistochemistry staining, and scored each sample according to the staining intensity (Figure 1) and staining area. Out of 751 stained colorectal cancer specimens, 689 (91.7%) showed positive PNPLA8 expression. These 751 samples were categorized into PNPLA8-low expression group and PNPLA8-high expression group, and the correlations between PNPLA8 expression and the clinicopathological parameters were analyzed (Table 2). A positive correlation was observed between high cytoplasmic PNPLA8 staining and M stage (P = 0.048). However, there were no significant correlations between PNPLA8 staining and other parameters (P > 0.05), including age, gender, CEA, CA199, tumor location, tumor size, primary histological type, primary differentiation, T stage, N stage, vascular invasion, and nerve invasion.

High PNPLA8 expression is associated with poor overall survival of CRC patients

To further substantiate the importance of high PNPLA8 expression in colorectal cancer progression, we compared the overall survival of CRC patients in our study cohort with differential PNPLA8 expression levels. The median follow-up OS of the CRC patients was 46.1 mo (IQR = 36.9-60.9). We found that PNPLA8 expression was stastically significantly associated with a shorter OS (HR 1.445; 43.1 mo for PNPLA8-low group *vs* 35.4 mo for PNPLA8-high group; P = 0.005) (Figure 2). Therefore, higher PNPLA8 expression could predict poor overall survival in patients with CRC, suggesting that PNPLA8 is a prognostic factor of CRC.

We then conducted further stratified analysis according to TNM stage of CRC patients. For CRC patients at Stage I and II, PNPLA8 expression was a significant prognostic factor (HR 2.578, $P < 0.01$; Figure 3A). For CRC patients at Stage III, OS did not show statistical differences among patients with different PNPLA8 expression levels (HR 1.061, $P = 0.083$; Figure 3B). For CRC patients at Stage IV, the patients with a higher PNPLA8 expression also had stastically significant shorter OS (HR 1.476, $P = 0.036$; Figure 3C). In stratified analysis according to tumor location, for patients with right-sided colon cancer (Figure 3E), PNPLA8 expression was not a significant prognostic factor ($P = 0.7057$). However, for patients with left-sided colon (Figure 3D) and rectal cancer (Figure 3F), the survival curves of two PNPLA8-expression groups showed stastically significant differences (HR 1.886, $P = 0.009$ for left-sided colon cancer; HR 1.583, $P = 0.035$ for rectal cancer).

PNPLA8 and several clinicopathological parameters are independent prognostic factors of CRC

Using univariate analysis, we found that CRC patients with a PNPLA8-high expression showed significantly differences when compared a PNPLA8-low expression in terms of multiple parameters, including CEA ($P < 0.001$), CA199 ($P < 0.001$), primary differentiation ($P = 0.02$), T stage ($P < 0.001$), N stage ($P < 0.001$), M stage ($P < 0.001$), vascular invasion ($P < 0.001$), and nerve invasion ($P < 0.001$) (Table 3). Therefore, multivariate analysis was performed using the Cox proportional hazards model for all of the significant variables examined in the univariate analysis. We found that PNPLA8 expression was proved to be a statistically significantly independent prognostic factor (HR 1.328, $P = 0.038$). In addition, CA199 (HR 1.548, $P = 0.004$), N stage (HR 1.701, $P < 0.001$), M stage (HR 4.862, $P < 0.001$) and Vascular invasion (HR 1.512, $P = 0.017$) (Table 3) were also proved to be independent factors.

DISCUSSION

In this study, a large cohort of real world was employed to evaluate the prognostic value of PNPLA8 in colorectal cancer patients. Patients with a higher PNPLA8 expression were confirmed to have a significantly impaired OS. Moreover, PNPLA8 expression was identified as a new independent prognostic factor for OS of CRC patients.

PNPLA8 is part of a diverse family of phospholipase A2 enzymes (PLA_2s) that hydrolyze the *sn*-2 substituent from membrane phospholipids to release a free fatty acid and a lysolipid[16,11]. These enzymes are ubiquitously expressed, and in contrast to secretory PLA_2s and cytosolic PLA_2s, do not require Ca^{2+} for either translocation or activity. Some of the first descriptions of $iPLA_2$ activity were in the mid- to late-1980s with the identification of a plasmalogen-selective $iPLA_2$in PNPLA8, when PNPLA8 was found to function as phospholipases and was better characterized[17]. During the past few years, knockout and transgenic mice for manipulated PNPLA8 expression have been established[18], and then studies using these gene-manipulated mice have provided models with which to elucidate the physiological and pathophysiological roles of PNPLA8. Recently, the mechanisms by which phospholipase A2 enzymes mediate lipid reprogramming and glycerophospholipid remodeling in cancer cells are illustrated[19,20]. PLA2s as the upstream regulators of the arachidonic acid cascade are generally high expressed and activated in various cancers[19,20]. Therefore, they are potential pharmacological targets and biomarkers in cancer.

Our findings are in line with previous reports showing that PNPLA8 expression is increased in colorectal cancer[21,12]. The ability of PNPLA8 to promote cell proliferation becomes prominent in the context of tumorigenesis. Several *in vitro* studies reveal a higher expression of PNPLA8 in stimulated immortal cell lines and that chemical inhibition or siRNAs-mediated PNPLA8 knockdown reduces proliferation and promotes apoptosis of the cells[22,23]. Subsequent studies targeting specific cancers suggest that PNPLA8 promotes cancer cell growth *via* signal transduction pathways involving epidermal growth factor receptors, mitogen-activated protein kinases, E3 ubiquitin-protein ligase Mdm^2, tumor suppressor protein p53, and cell cycle regulator

p21[24,25]. Therefore, there is increasing support for a role of PNPLA8 and PNPLA8-associated phospholipase A in promoting cancer cell proliferation and metastasis, which plausibly provides the molecular mechanisms underlying our finding of PNPLA8 as a novel prognostic factor for colorectal cancer.

However, a couple of limitations of this study must be noticed. First, our study is a retrospective one. To further validate our conclusion, a prospective study with data from multiple centers is necessary, especially for patients with left-sided colon and rectal cancer as well as Stage I and II patients. Second, the scores for PNPLA8 protein intensity and area were not determined in a fully automated way, resulting in potential artificial errors due to possible human bias. Third, in-depth *in vitro* and *in vivo* experiments are urgently needed to unveil the hidden mechanisms of PNPLA8 in colorectal cancer.

CONCLUSION

In summary, PNPLA8 was identified as a new independent prognostic factor for colorectal cancer. CRC with a high PNPLA8 expression conferred survival impairment. Our findings provide critical insights into aiding the individualized treatment of patients with colorectal cancer.

ARTICLE HIGHLIGHTS

Research background

The role of patatin like phospholipase domain containing 8 (PNPLA8) in colorectal cancer progression is unclarified.

Research motivation

The research motivation is to explore the prognostic effects of PNPLA8 expression in colorectal cancer.

Research objectives

These findings suggest that PNPLA8 is a potential target for clinical colorectal cancer therapy, providing important insights to help personalize therapy for patients with colorectal cancer.

Research methods

PNPLA8 expression in tumor samples was evaluated by immunohistochemistry staining, and semi-quantitated with immunoreactive scores.

Research results

Colorectal cancer (CRC) patients with a high PNPLA8 expression indicated poor overall survival (OS) (median OS = 35.3, P = 0.005). The multivariate analysis also confirmed that high PNPLA8 expression was a significantly independent prognostic factor for overall survival (hazard ratio HR = 1.328, 95%CI 1.016-1.734, P = 0.038).

Research conclusions

PNPLA8 is a novel independent prognostic factor for CRC. These findings indicate PNPLA8 as a potential target in clinical CRC management.

Research perspectives

These findings suggest that PNPLA8 is a potential target for clinical colorectal cancer therapy, providing important insights to help personalize therapy for patients with colorectal cancer.

REFERENCES

1 **Bao,** S., Song, H., Tan, M., Wohltmann, M., Ladenson, J. H., & Turk, J. (2012). Group VIB Phospholipase A(2) promotes proliferation of INS-1 insulinoma cells and attenuates lipid peroxidation and apoptosis induced by inflammatory cytokines and oxidant agents. Oxid Med Cell Longev, 2012, 989372. DOI:10.1155/2012/989372

2 **Biller,** L. H., & Schrag, D. (2021). Diagnosis and Treatment of Metastatic Colorectal Cancer: A Review. Jama, 325(7), 669-685. DOI:10.1001/jama.2021.0106

3 **Blanchard,** H., Taha, A. Y., Cheon, Y., Kim, H. W., Turk, J., & Rapoport, S. I. (2014). iPLA2β knockout mouse, a genetic model for progressive human motor disorders, develops age-related neuropathology. Neurochem Res, 39(8), 1522-1532. DOI:10.1007/s11064-014-1342-y

4 **amp,** R. L., Dolled-Filhart, M., & Rimm, D. L. (2004). X-tile: a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization. Clin Cancer Res, 10(21), 7252-7259. DOI:10.1158/1078-0432.Ccr-04-0713

5 **ohen,** D., Papillon, J., Aoudjit, L., Li, H., Cybulsky, A. V., & Takano, T. (2008). Role of calcium-independent phospholipase A2 in complement-mediated glomerular epithelial cell injury. Am J Physiol Renal Physiol, 294(3), F469-479. DOI:10.1152/ajprenal.00372.2007

6 **ekker,** E., Tanis, P. J., Vleugels, J. L. A., Kasi, P. M., & Wallace, M. B. (2019). Colorectal cancer. Lancet, 394(10207), 1467-1480. DOI:10.1016/s0140-6736(19)32319-0

7 **ennis,** E. A., Cao, J., Hsu, Y. H., Magrioti, V., & Kokotos, G. (2011). Phospholipase A2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. Chem Rev, 111(10), 6130-6185. DOI:10.1021/cr200085w

8 **ara,** S., Yoda, E., Sasaki, Y., Nakatani, Y., & Kuwata, H. (2019). Calcium-independent phospholipase A(2)γ (iPLA(2)γ) and its roles in cellular functions and diseases. Biochim Biophys Acta Mol Cell Biol Lipids, 1864(6), 861-868. DOI:10.1016/j.bbalip.2018.10.009

9 **ooks,** S. B., & Cummings, B. S. (2008). Role of Ca2+-independent phospholipase A2 in cell growth and signaling. Biochem Pharmacol, 76(9), 1059-1067. DOI:10.1016/j.bcp.2008.07.044

10 **aburek,** M., Pruchova, P., Holendova, B., Galkin, A., & Jezek, P. (2021). Antioxidant Synergy of Mitochondrial Phospholipase PNPLA8/iPLA2gamma with Fatty Acid-Conducting SLC25 Gene Family Transporters. Antioxidants (Basel), 10(5). DOI:10.3390/antiox10050678

11 **i,** M., Ren, L., Lv, Y., Lao, X., Feng, Q., Tang, W., . . . Xu, J. (2020). Small Nuclear Ribonucleoprotein Polypeptide N Accelerates Malignant Progression and Poor Prognosis in Colorectal Cancer Transcriptionally Regulated by E2F8. Front Oncol, 10, 561287. DOI:10.3389/fonc.2020.561287

12 **han,** S. A., & Ilies, M. A. (2023). The Phospholipase A2 Superfamily: Structure, Isozymes, Catalysis, Physiologic and Pathologic Roles. Int J Mol Sci, 24(2). DOI:10.3390/ijms24021353

13 **i,** N., Lu, B., Luo, C., Cai, J., Lu, M., Zhang, Y., . . . Dai, M. (2021). Incidence, mortality, survival, risk factor and screening of colorectal cancer: A comparison among China, Europe, and northern America. Cancer Lett, 522, 255-268. DOI:10.1016/j.canlet.2021.09.034

14 **iu,** G. Y., Moon, S. H., Jenkins, C. M., Li, M., Sims, H. F., Guan, S., & Gross, R. W. (2017). The phospholipase iPLA(2)gamma is a major mediator releasing oxidized aliphatic chains from cardiolipin, integrating mitochondrial bioenergetics and signaling. J Biol Chem, 292(25), 10672-10684. DOI:10.1074/jbc.M117.783068

15 **oon,** S. H., Jenkins, C. M., Liu, X., Guan, S., Mancuso, D. J., & Gross, R. W. (2012). Activation of mitochondrial calcium-independent phospholipase A2γ (iPLA2γ) by divalent cations mediating arachidonate release and production of downstream eicosanoids. J Biol Chem, 287(18), 14880-14895. DOI:10.1074/jbc.M111.336776

16 **urakami,** M., Masuda, S., Ueda-Semmyo, K., Yoda, E., Kuwata, H., Takanezawa, Y., . . . Kudo, I. (2005). Group VIB Ca2+-independent phospholipase A2gamma promotes cellular membrane hydrolysis and prostaglandin production in a manner distinct from other intracellular phospholipases A2. J Biol Chem, 280(14), 14028-14041. DOI:10.1074/jbc.M413766200

17 **urase,** R., Taketomi, Y., Miki, Y., Nishito, Y., Saito, M., Fukami, K., . . . Murakami, M. (2017). Group III phospholipase A(2) promotes colitis and colorectal cancer. Sci Rep, 7(1), 12261. DOI:10.1038/s41598-017-12434-z

18 **appi,** A., Nasti, G., Romano, C., Berretta, M., & Ottaiano, A. (2020). Metastatic Colorectal Cancer: Prognostic and Predictive Factors. Curr Med Chem, 27(17), 2779-2791. DOI:10.2174/0929867326666190620110732

19 **eng,** Z., Chang, Y., Fan, J., Ji, W., & Su, C. (2021). Phospholipase A2 superfamily in cancer. Cancer Lett, 497, 165-177. DOI:10.1016/j.canlet.2020.10.021

20 **amanadham,** S., Ali, T., Ashley, J. W., Bone, R. N., Hancock, W. D., & Lei, X. (2015). Calcium-independent phospholipases A2 and their roles in biological processes and diseases. J Lipid Res, 56(9), 1643-1668. DOI:10.1194/jlr.R058701

21 **aunders,** C. J., Moon, S. H., Liu, X., Thiffault, I., Coffman, K., LePichon, J. B., . . . Gross, R. W. (2015). Loss of function variants in human PNPLA8 encoding calcium-independent phospholipase A2 gamma recapitulate the mitochondriopathy of the homologous null mouse. Hum Mutat, 36(3), 301-306. DOI:10.1002/humu.22743

22 **cott,** K. F., Sajinovic, M., Hein, J., Nixdorf, S., Galettis, P., Liauw, W., . . . Russell, P. J. (2010). Emerging roles for phospholipase A2 enzymes in cancer. Biochimie, 92(6), 601-610. DOI:10.1016/j.biochi.2010.03.019

23 **iegel,** R. L., Miller, K. D., Fuchs, H. E., & Jemal, A. (2022). Cancer statistics, 2022. CA Cancer J Clin, 72(1), 7-33. DOI:10.3322/caac.21708

24 **aketo,** M. M., & Sonoshita, M. (2002). Phospolipase A2 and apoptosis. Biochim Biophys Acta, 1585(2-3), 72-76. DOI:10.1016/s1388-1981(02)00326-8

25 **hu,** D., Xia, J., Gu, Y., Lin, J., Ding, K., Zhou, B., . . . Xu, J. (2021). Preoperative Hepatic and Regional Arterial Chemotherapy in Patients Who Underwent Curative Colorectal Cancer Resection: A Prospective, Multi-center, Randomized Controlled Trial. Ann Surg, 273(6), 1066-1075. DOI:10.1097/sla.0000000000004558

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