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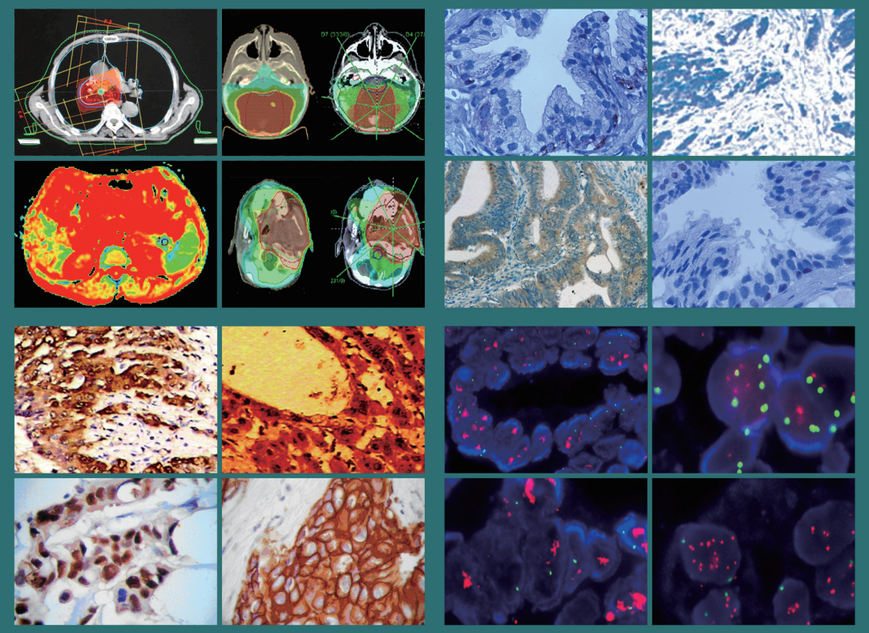

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Aims & Scope

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REVIEW ARTICLE

Biochemical overview of the recent findings on the correlation between viral hepatitis and its related hepatocellular carcinoma

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Abstract

As one of the most common primary liver cancers, hepatocellular carcinoma (HCC) usually occurs in the presence of inflammation. Compared to other risk factors such as alcohol abuse, aflatoxin, and obesity, virus-induced hepatitis can be effectively prevented by vaccines. For the past several decades, HCC has been believed to be closely related to viral infections although no comprehensive mechanism was established regarding the contribution of viral hepatitis toward HCC. Recent studies have shown that viral infection plays multiple roles in the process of carcinogenesis by causing an increase in genomic instability, cancer-promoting genetic mutations, signal pathway interruption, and tumor suppressor gene inhibition. Sorafenib has become a novel option for HCC patients, especially those who are in advanced disease stage for which conventional treatment methods are not recommended. Future studies should focus more on novel targeted drugs which can be adopted as alternatives to sorafenib or as second-line drugs after the failure of sorafenib

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Key words: HBV; HCV; hepatocellular carcinoma (HCC); correlation

According to Dr. Jeffrey Stanaway, worldwide, 1.45 million lives were lost to viral hepatitis in 2013, which was significantly greater than the 0.89 million deaths in 1990. Among all viral hepatitis-related morbidity cases, the prevalence of hepatitis C infection-related morbidity was the highest [1]. It has been well known that hepatitis-related mortality is influenced by geographical variation; Oceania, western-sub-Saharan Africa, and central Asia have the highest mortality rate. Not surprisingly, East Asia and South Asia have the highest absolute numbers because of their large population [2]. Hepatitis-related cirrhosis is often observed in viral hepatitis patients as the sequential progression. Scientists have long believed that there is a close association between hepatitis-related cirrhosis and hepatocellular carcinoma (HCC) although there is no comprehensive understanding yet. HCC is the most common primary liver cancer with a complex etiology and has mechanisms that make early clinical detection and treatment of this disease challenging. As one of the most critical risk factors for HCC, viral hepatitis and hepatitis-related cirrhosis deserve more attention in future studies. The aim of this paper was to review biomolecular characteristics of viral hepatitis and some recently proposed findings with regard to the correlation between viral hepatitis and its associated HCC along with discussing potential treatment and future perspectives.

Pathophysiology of viral hepatitis and viral hepatitis-related responses

Molecular biology of HBV and HCV infections

The four overlapping reading frames are encoded by HBV viral genome which includes the following ORFs: S, C, P, and X. The viral surface proteins and HBsAg are encoded by S ORF. The core gene (C) is responsible for the synthesis of either hepatitis B core antigenor (HBcAg) hepatitis B e-antigen (HBeAg). The large protein polymerase (pol), which facilitates viral replication, is encoded by P ORF. The viral X ORF encodes HBxAg which is vital for signal transduction, transcription activation, DNA repair, and inhibition of protein degradation [3]. HBV invades the cell by binding to its receptor, namely

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the bile acid pump sodium-taurocholate co-transporting polypeptide A (NTCP1), on the cell surface and releases its DNA into the target cell nucleus. After converting to a cccDNA minichromosome, HBV transcription can be performed using cccDNA as the template [4]. The transcription is driven by promoters and regulated by transcription factors, and reverse transcription is completed in the presence of viral polymerase using pre-genomic RNA as the template. The relaxed circular DNA (rcDNA) of the virus is formed after a complementary strand is created. Binding with viral polymerase enables rcDNA packaging and new virus formation [5].

Because Hepatitis C virus is an RNA virus different from HBV, the positive stranded RNA of HCV is cleaved into 10 proteins by its host cell and viral proteases after its translation [core protein, envelope 1 and 2 (identified as structural proteins, and p7 as well as NS2, NS3, NS4A, NS4B, NS5A, and NS5B (classified as non-structural proteins)] through a cap-independent mechanism mediated by HCV IRES. Similarly, the replication of HCV is carried out by the synthesis of positive and negative strands of HCV RNA, which serve as the template. The replication is mainly regulated by cis-elements. Additionally, some long-range RNA-RNA interactions (LRIs) and liver-specific microRNA-122 (miR-122) play significant roles in viral RNA translation and replication [6].

It has long been believed that chronic hepatocytic injury is induced by immune cell activity in cases of both HBV and HCV infections rather than via a cytopathic effect. In both HBV and HCV infections, liver injury is usually caused by the inflammatory response. Hepatocytes release proinflammatory cytokines such as TNF-á, IL-6, IL-1, and IL-18, and initiate the accumulation of granulocytes, mononuclear phagocytes, and macrophages at the site of inflammation as well as facilitate the elimination of the invading virus. Because of the over-reactive immune response against the virus, several hepatocytes can accidentally get injure during the inflammation process [7]. In patients without an efficient adaptive immune system, acute viral infection can develop into a chronic infection. During the prolonged process of inflammation, multiple types of hepatic cells are involved. The hepatic stellate cells (HSCs) are normally located in the space between hepatocytes and endothelial cells. The quiescent HSC mainly plays a role in vitamin A storage. When HSCs are activated during the inflammatory response, extracellular matrix and collagen production by these cells is triggered by releasing TGF-â which is a strong inducer of fibrogenesis. Moreover, the pattern of adhesion molecule expression in endothelial cells is altered during chronic inflammation [8]. Similar to endothelial cells and HSC, Kupffer cells also release cytokines and chemokines during inflammation and exhibit changes in the expression of adhesion molecules.

Immunologically, cytotoxic T cells are involved in viral elimination by recognizing the viral antigens presented on the host cell surface, and this mechanism is responsible for the clearance of a majority of viruses during an acute infection. Other immune cells also play roles in the inflammation process. The pit cells (a type of NK cells) respond to inflammation as well. They produce IFN-ã, TNF-á, and cytokines to destroy the abnormal cells (malignant or infected cells). Neutrophils are attracted by cytokines released from Kupffer cells and hepatocytes and migrate from other parts of the body to hepatic tissues. The net result is to increase inflammation and intensify inflammatory injury caused to the liver [9].

During inflammation, multiple inflammatory factors are released by cells. Moderate cell necrosis and extracellular matrix damage will follow inflammation and cell injury. Consequently, fibrogenesis occurs for repairing the injured tissues and replacing them with granulation tissues. Once reaching an advanced level, fibrogenesis progresses to cirrhosis, which is a later stage of fibrosis; during this stage, regenerative nodules and fibrotic bands are formed in the perisinusoidal space to give the liver a “bumpy” and stiff texture [10].

Involvement of molecular pathogenesis in oncogenesis

Cirrhosis is one of the most important risk factor of HCC and exhibits a close correlation with HCC, which is the cancer responsible for the highest morbidity rate among all primary liver malignancies worldwide. Among 80%–90% of HCC cases, HBV or HCV infection occurs, and the 5-year cumulative risk of HCC development in virus-induced cirrhosis patients varies from 5% to 30% [11]. According to Fattovich, the estimated incidence rate of HCC in East Asia among inactive HBV carriers is 0.2 per 100 person-years and 0.6 per 100 person-years among chronic HBV infection patients without cirrhosis. Additionally, the incidence of HCC significantly increases in chronic HBV infection patients with cirrhosis [12]. Moreover, the positive correlation between cirrhosis severity and HCC incidence has been proved by another cohort study conducted in Taiwan from 1997 to 2011 [13]. The inflammatory response, oxidative stress, and fibrosis may contribute to hepatocyte transformation and HCC development. However, the links between virus-induced hepatitis and HCC have not been clearly understood. Thus far, several mechanisms have been suggested for the initiation of HBV-induced HCC. First, HBV-induced chronic inflammation and hepatocyte regeneration could cause genetic alterations. Second, genomic instability and alteration of cancer-related gene expression can be induced by HBV integration; this

would finally lead to hepatocarcinogenesis. In a previous study, HBV integration events were observed in some common host genes including KMT2B, FN1, and TERT [14]. In recent years, multiple roles of HBV-X protein in the process of oncogenesis have been widely recognized. HBx is involved in cellular signal pathways, transcription regulations, cell cycle mediation, DNA repair, apoptosis, and genetic stability through interactions with host factors [15]. However, the mechanism of HCC induction by HBV remains unclear. The development of HBV-induced HCC might be owing to the combined actions of all mechanisms discussed previously. In mice models, the expression of HBx in liver tissues results in increased susceptibility to hepatocarcinogens [16]. HBx can bind to p53 and inhibit its activity as a tumor suppressor through a complicated mechanism. HBx can also bind to NF-êB (nuclear factor) and promote HCC cell invasion and metastasis [17]. Additionally, HBx also plays a role in the regulation of histone modification. For instance, HBx can interfere with gene transcription and stimulate tumorigenesis through interaction with CBP/p300 histone acetyl-transferase complex, and this phenomenon can often be observed in HBV-related HCC [18].

HBV and HCV infections are reportedly related to elevated levels of â-catenin, which is an important part of Wnt/â-catenin signaling pathways and considered to be significant in cancer development. An abnormality in the Wnt/â-catenin signaling pathway increases the incidence of cancer [19].

Furthermore, some mutations seem to be closely related to viral infection and HCC. In HBV virus, mutations can occur in multiple gene regions because HBV reverse transcriptase does not exhibit a proofreading function. Pres/S, preC/C, polymerase/reverse transcriptase, and X ORF gene sites are the most common regions undergoing mutations that are closely related to the development of HCC [20]. Somatic mutations in protein coding genes such as Wnt (CTNNB1) and TP53 are most commonly encountered during HCC infection, and mutation is observed more frequently in TP53 during HBV infection [21]. TERT promoter mutations were prevalent in 59% of the tested human HCC samples, and this mutation was reportedly involved in malignantly transforming hepatocellular adenoma into HCC [22]. Moreover, the frequency of mutation in the TERT promoter increased with an increasing grade of dysplastic nodules (low grade too high grade) and peaked during the early stages of HCCs. Besides, TERT promoter mutation is recognized as the earliest somatic alteration and believed to be a novel predictive biomarker for oncogenesis stemming from cirrhosis [23]. Moreover, telomerase promoter mutations can cause telomerase overexpression and telomere shortening. Telomere shortening causes cell senescence or apoptosis and decreases the regeneration ability of liver cells, consequently resulting in fibrosis and cirrhosis, which is a precursor to HCC [24].

Dysregulated DNA methylation is another factor for HCC in chronic HBV and HCV infections. Dysregulation of DNA methylation includes hypomethylation and hypermethylation. Hypomethylation influences structural-nuclear function, and hypermethylation can lead to the inhibition of tumor suppressor genes. Specifically, methylated genes which involved signaling pathways such as RAS/RAF/ERK and Wnt/â-catenin pathways are observed more often in HCV-induced HCC. Methylation of GSTP1 and E-cadherin promoters preferentially occurs in HBV-induced HCC [25].

Unlike HBV infection, HCV infection does not integrate into the host genome. However, inflammation, steatosis, oxidative stress, and progressive fibrosis in HCV infection ultimately lead to HCC development. HCV infection indirectly triggers hepatocarcinogenesis. Moreover, it directly affects physical processes including metabolism, DNA repair, and apoptosis by altering cellular pathways [26]. HCV can contribute to the breaking of double-stranded DNA, thereby increasing the possibility of gene mutations. Such mutations can be found in immunoglobulin genes, BCL-6, TP53, and oncogene CTNNB1 (encodes â-catenin in WNT pathway) [27]. HCV-infected cells showed elevated â-catenin levels, although its significance is not clear yet [28]. E1, E2, NS3, and NS5 as core proteins of HCV are thought to be crucial for tumorigenesis because they modulate cell signaling by interacting with several cellular proteins [29]. Additionally, as a viral product protein, HCV capsid is involved in the process of cell proliferation and modulates cellular gene functions. A previous study has reported that transfection of the *ras* oncogene causes rat embryo fibroblasts to be transformed into a malignant phenotype by the human *c-myc* promoter gene in the presence of HCV capsid [30]. Moreover, Nishiguchi et al emphasized the correlation between HCV infection and HCC in the study investigating the effects of IFN-á on patients with HCV and cirrhosis. In that study, only 4% of the patients in the IFN-á group develop HCC as opposed to 38% of patients in the placebo group [31].

Additionally, HCC occurrence is not correlated only with viral infection. Cirrhosis itself is also an independent factor which increases the risks of HCC. A noteworthy link between HCC incidence and severity of virus-induced cirrhosis is proved by the data collected from 1997–2011 in Taiwan. It has been revealed that cirrhosis patients with other complications have a significantly higher risk of HCC compared with cirrhosis patients without any complications [32]. Other than that, underlying cirrhosis in HCC patients makes the disease treatment more challenging and also significantly reduces the survival rate because of its association with several

complex and critical complications. The irreversibility and complicated complications coming along with cirrhosis gives a moderate survival rate of patients.

Discussion and future perspectives

Currently, the first-line treatment for patients with solitary tumors is hepatic resection (with well-preserved liver function). Specifically, normal serum bilirubin levels with either an HVPG of ≤ 10 mm Hg or a platelet count of $\geq 100\times10^9$/L. For patients with poorer liver function and at a Child–Pugh stage A or B, local ablation is suitable for tumors measuring < 3 cm in size. Other than that, liver transplantation is the optimal choice for patients with single tumors of size ≤ 5 cm or up to three tumors measuring ≤ 3 cm in size. For patients with compensated liver disease and large or multifocal HCC without vascular invasion or extrahepatic spread, transarterial chemoembolization (TACE) is considered prior to other alternatives [33].

Nowadays, the multi—kinase inhibitor sorafenib has become the standard therapy in advanced HCC and Child–Pugh class A according to the current guidelines. However, novel drugs are needed for advanced HCC patients as alternatives to sorafenib failure. Antibody against cell death protein (PD-1) as an immune checkpoint inhibitor showed an overall response rate of 19% in HCC patients in a phase I study reported in 2015. Anti-PD-1 (nivolumab) achieves the goal of tumor attack through T cell re-storage and prevents cancer immunotolerance [34]. Besides, lenvatinib has been discovered to be an effective angiogenesis inhibitor which primarily suppresses tumor growth by blocking vascular endothelial growth factor receptors (VEGFR1, VEGFR2, VEGFR3), fibroblast growth factor receptors (FGFR1, FGFR2, FGFR3, and FGFR4), KIT, and RET [35].

Furthermore, recent studies have identified that miR-214 is a tumor suppressor, which significantly inhibits on cell growth, cell proliferation, and colony forming ability as well as decreases the levels of â-catenin protein and its downstream proteins including Cyclin D1, c-Myc and TCF-1 [36]. This finding may shed light on a novel perspective for future HCC treatment.

Conclusion

Although HCC is a disease caused by many factors and characterized by its great difficulty in treatment, viral infection is the controllable factor that can be prevented effectively through vaccination and health education; these strategies can greatly lower the incidence of virus-induced HCC, thereby decreasing the mortality. Mechanisms underlying the development of HCC caused by the association of viral infection (HBV and HCV) with cirrhosis have not been clearly understood. However, the effects of viral infection on gene mutations and signal pathways are widely recognized. Moreover, the vital role of HBx in oncogenesis is has been recently proved. Traditional options of HCC treatment are mainly focused on hepatic resection, local ablation, and TACE according to concrete states of disease. Sorafenib is the only targeted drug recommended by the current guidelines for HCC treatment.

Conflict of interest

The authors confirm that this article has no conflict of interest.

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ORIGINAL ARTICLE

Local definitive intensity-modulated radiation therapy recommended for patients initially diagnosed with nasopharyngeal carcinoma with distant metastasis after an effective systemic chemotherapy*

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Abstract

Objective The aim of the study was to propose a hypothesis that local definitive intensity-modulated radiation therapy (IMRT) should be recommended for initially diagnosed metastatic nasopharyngeal carcinoma (NPC) and demonstrate its feasibility.

Methods Recently published papers on local definitive radiotherapy for initially diagnosed metastatic NPC were reviewed to propose a hypothesis.

Results Several studies revealed the survival benefits of adding local definitive radiotherapy to the systemic chemotherapy in patients initially diagnosed with metastatic NPC.

Conclusion We suggested that local definitive IMRT should be recommended in patients initially diagnosed with NPC with distant metastasis after an effective systemic chemotherapy, which may possibly prolong their survival time and potentially treat the disease.

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Key words: nasopharyngeal carcinoma (NPC); metastasis; intensity-modulated radiation therapy (IMRT)

Nasopharyngeal carcinoma (NPC) is one of the most common head and neck cancers in southern China. In endemic areas, the NPC incidence is approximately 25–30 per 1 000 000 persons per year [1]. About 4% of patients are initially diagnosed with distant metastasis, especially those with locally advanced disease [2].

Despite the significant progress in long-term disease control in patients with early stage and locally advanced disease, metastatic NPC is still conventionally regarded as incurable. Palliative chemotherapy is the primary and frequently used therapeutic strategy for metastatic NPC. Although chemotherapy yields high objective response rates, the median survival in patients with metastatic NPC is merely 12–20 months after various chemotherapy regimens [3]. Most patients who undergo chemotherapy have persistent locoregional disease. This condition often accelerates disease progression after the first-line systemic chemotherapy. The 5-year overall survival (OS) rate in patients with metastatic NPC is only 20%, which is in contrast to > 80% in patients without metastasis [4]. Therefore, managing NPC patients with metastasis remains a therapeutic challenge.

No consensus is currently available among oncologists with regard to the optimum treatment modality for metastatic patients with initially diagnosed NPC. Radiotherapy has been the mainstay treatment for patients with nonmetastatic NPC; however, its role in patients with metastatic disease remains controversial [5].

The addition of local therapy, such as radiation, to the systemic chemotherapy for metastatic cancer has been practiced for metastatic rectal, esophageal, breast, and lung cancers [6–9]. With the rapidly emerging oncologic concept of using local treatment for limited metastatic disease, several studies recently reported the survival benefit of adding local definitive radiotherapy to systemic chemotherapy in metastatic patients with initially

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diagnosed NPC [10–19].

Hypothesis

In this study, we hypothesized that local definitive intensity-modulated radiation therapy (IMRT) should be recommended after an effective systemic chemotherapy for patients with initially diagnosed NPC who developed distant metastasis. The current systemic chemotherapy merely leads to a short median survival time of 12–20 months, and the radiotherapy dose for the primary tumor and cervical disease is often palliative. This condition fails to prolong the survival time. Recent studies show that patients diagnosed with NPC with distant metastasis who received curative local radiotherapy after an effective systemic chemotherapy had improved survival compared with those who did not.

Discussion and conclusion

Radiotherapy is usually the mainstay treatment for patients with nonmetastatic NPC because of its anatomic location and relatively high radiosensitivity. However, the role of local radiotherapy in NPC patients with metastasis remains unclear.

First, many oncologists consider local radiotherapy unnecessary because of distant metastasis, which signifies clinical incurability. Palliative chemotherapy is currently the most frequently used therapeutic strategy. The single systemic chemotherapy treatment modality for metastatic NPC is largely insufficient, especially for diseases with primary bulky tumor and those that occur close to the critical organs, such as the brainstem, carotid arteries, and optic chiasm. Persistent locoregional disease is often observed in these patients after receiving systemic chemotherapy treatment alone. With disease progression, the primary tumor may cause severe symptoms and complications, such as massive hematuria, severe headache, hearing impairment, blindness, and brainstem injury. These factors will compromise the patients' quality of life and even lead to death. A subgroup of patients with NPC will eventually die of local failure of treatment after a systemic chemotherapy because the oncologists are not concerned of the distant foci [20]. In addition, the current NPC tumor-node-metastasis classification has drawbacks in the M stage, which is still a "catch-all" classification in patients who differ in terms of the specific organs involved and the number and location of lesions in each organ. Subdividing the M1 stage in patients with metastatic NPC may help oncologists stratify patients according to prognosis and guide treatment decisions. Many reports showed that a single lesion confined to an isolated organ or oligometastasis is associated with prolonged survival time compared with widespread metastatic lesions in isolated or multiple locations [13–16]. Moreover, many reports showed that selective patients with NPC and limited metastatic lesions are potentially treated using the appropriate combination of systemic chemotherapy and definitive local radiotherapy [10–19]. Zou *et al* successfully subdivided the M1 stage into three groups: M1a, oligometastasis without liver involvement; M1b, multiple metastases without liver involvement; and M1c, liver involvement irrespective of metastatic lesions. The 3-year OS was 54.5%–72.8% for M1a, 34.3%–41.6% for M1b, and 22.6%–23.6% for M1c [13]. Shen *et al* developed an M categorization system based on factors related to the prognosis of patients with metastatic NPC. They defined the following groups based on liver involvement and number of metastatic lesions: M1a, single lesion confined to an isolated organ or location except the liver, which had the best prognosis; M1b, single lesion in the liver and/or multiple lesions in any organs or locations except the liver, which had the modest prognosis; and M1c, multiple lesions in the liver, which had the worst prognosis [16]. These studies indicated that patients with more than three metastatic sites had significantly poorer OS than those with three or fewer metastatic sites. Compared with liver metastases, lung or bone metastasis was demonstrated as a positive factor of survival, which may lead to long-term survival. The reason of poor survival in liver metastasis may be related to the rich blood supply of the liver and the low response rate to the systemic chemotherapy. Therefore, local radiotherapy should be included after effective systemic chemotherapy, especially in patients with single or limited metastatic sites without liver involvement.

Second, several oncologists are concerned about radiotherapy-related toxicities, which might compromise the patients' quality of life or even shorten the patients' survival time. However, this treatment concept and concern are from the conventional two-dimensional (2D) radiotherapy era. With the development of radiobiology and radiophysics, IMRT has been used worldwide because of its improved tumor target conformity, good local control, and low radiation side effects [21]. The conventional 2D radiotherapy has already been replaced by IMRT in NPC treatment. Therefore, the role of IMRT in patients newly diagnosed with metastatic NPC should be re-evaluated.

The significance of local definitive radiotherapy for patients initially diagnosed with metastatic NPC has been evaluated in many studies. Verma *et al* [10] used the National Cancer Database (NCDB) to analyze the outcomes in patients with metastatic NPC who received chemoradiotherapy versus those who received chemotherapy alone. Among the 555 patients, 296 (53%) underwent chemotherapy alone, and 259 (47%) received definitive chemoradiotherapy. The median OS rates in

the chemotherapy alone and chemoradiotherapy groups were 13.7 and 25.8 months, respectively ($P < 0.01$). With the multivariate analysis, the treatment with additional radiotherapy was independently predicted to significantly improve the OS time ($P < 0.01$). Rusthoven *et al* [11] also used the NCDB to evaluate the outcomes in patients with metastatic NPC receiving chemotherapy with and without local radiotherapy. In this largest reported analysis on chemotherapy with and without local radiotherapy for metastatic NPC, 718 NPC patients with metastasis were identified (39% chemotherapy alone and 61% chemotherapy + radiotherapy). At a median follow-up of 4.4 years, radiotherapy was found to be associated with improved survival time (median OS of 21.4 vs. 15.5 months; 5-year OS of 28% vs. 10%; $P < 0.001$). Long-term survival of > 10 years was only observed in the radiotherapy group. This result supported the strategies incorporating the local radiotherapy with chemotherapy for metastatic NPC. Hu *et al* [12] used the Surveillance Epidemiology and End Results database to examine the role of radiotherapy in treating metastatic NPC and identified 679 patients (66% chemotherapy + radiotherapy and 34% chemotherapy alone). Radiotherapy was associated with significantly improved OS [hazard ratio (HR): 0.50, $P < 0.001$] and cancer-specific survival (HR: 0.50, $P < 0.001$).

Third, in clinical practice, some oncologists merely provide palliative radiation dose to the primary tumor of NPC with distant metastasis. Palliative radiotherapy aims to control local symptom and improve the quality of life but not prolong the survival time. In the analysis of 718 NPC patients with metastatic reported by Rusthoven *et al* [11], radiotherapy dose was found to be an independent prognostic factor both as a continuous and categorical variable, with OS benefits observed among patients receiving ≥ 50 Gy. Patients receiving ≥ 70 Gy achieved the longest survival time. In a retrospective study by Hu *et al* [12], most patients with distant metastasis completed the full course of curative dose of IMRT (> 70 Gy) with estimated median OS time of 31.2 months. These studies indicated that using definitive IMRT combined with systemic chemotherapy to treat primary tumor might prolong the survival time in patients newly diagnosed with metastatic NPC.

The mechanisms underlying the survival benefit of the local definitive radiotherapy on metastatic NPC remains unclear. First, eliminating the primary tumor burden of NPC close to the critical organs could reduce the probability of death by uncontrolled local disease progression. Second, the primary tumor volume is closely related with survival rates in NPC and is a significant prognostic indicator of NPC treatment [22–25]. Local definitive radiotherapy reduces the primary tumor volume and leads to excellent local disease control and further survival benefit. Finally, other potential mechanisms in favor of radiotherapy for primary tumors could be the removal of immunosuppressive cytokines and enhancement of immune recognition [26].

In conclusion, we suggested that local definitive IMRT should be recommended in patients with initially diagnosed NPC with distant metastasis after an effective systemic chemotherapy to possibly prolong their survival time and potentially treat the disease.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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ORIGINAL ARTICLE

Role of serum lactate dehydrogenase levels in evaluating efficacy of interventional therapy for hepatocellular carcinoma

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Abstract

Objective The aim of the study was to investigate the role of serum lactate dehydrogenase (LDH) levels in evaluating the efficacy of transcatheter arterial chemoembolization (TACE) for primary liver cancer.

Methods A total of 52 patients with liver cancer admitted in our hospital (Huangzhou District People's Hospital, Huanggang, China) from June 2015 to December 2017 were selected and divided into control group (LDH of ≤ 450 U/L, n = 26) and observation group (LDH of > 450 U/L, n = 26), based on the pretreatment level of LDH. Based on the changes in serum LDH levels before and after treatment, patients were classified into two groups: LDH increased group (22 cases) and LDH decreased group (30 cases). The relationship between LDH levels and efficacy of TACE treatment was analyzed in the four groups retrospectively.

Results No significant difference was seen in the clinical characteristics (gender, median age, performance status Eastern Cooperative Oncology Group, and staging system) between the control and observation groups. The efficacy rate in the control group was 57.7%, whereas that in the observation group was 42.3% (P > 0.05). The 1-year survival rate in the observation group was 53.8% and that in the control group was 84.6% (P < 0.05).

Conclusion Serum LDH levels may be of clinical value in evaluating the efficacy of TACE in patients with hepatocellular carcinoma.

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Key words: primary hepatocellular carcinoma (HCC); lactate dehydrogenase (LDH); interventional therapy; prognosis

Primary hepatocellular carcinoma (HCC) is one of the most common malignant tumors in the liver, and its incidence increases with age. HCC has become the fifth leading cause of malignant tumors worldwide, and is the leading cause of cancer-related deaths after lung and gastric cancers [1–2]. The 5-year survival rate following surgical resection and transplantation ranges from 60%-70%. Therefore, surgical treatment is the first choice in patients with HCC indications [3].

However, most patients are diagnosed with intermediate or late HCC at the time of treatment and cannot be treated with radical therapy. Therefore, these patients need to consider palliative treatment. Common palliative treatments include arterial chemoembolization (TACE), radiotherapy, radiofrequency ablation, targeted therapy, chemotherapy, and traditional Chinese medicine [4–5]. In recent years, TACE has become one of the common treatment methods, but its clinical effect is still not satisfactory, and only some patients experience positive effects. At present, studies on the molecular mechanisms underlying the poor efficacy of TACE are ongoing.

Studies have shown that the adaptive regulation of hypoxia in the tumor tissue may be a key factor for poor efficacy [6]. Hypoxia can induce neovascularization and lead to therapeutic tolerance in cancer cells. In addition, evidence that hypoxia may promote the development of cancer is also increasing. The energy metabolism of cancer cells is significantly different from that of the normal tissues. Cancer cells maintain high aerobic glycolysis rates and produce high levels of lactic acid and pyruvate, a phenomenon known as the Warburg effect [7]. Lactate dehydrogenase (LDH) is a glycolytic enzyme

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consisting of four polypeptide chains. Each chain is encoded by different genes (M and H) that exist in various human tissues and tumors. LDH is the key enzyme for converting pyruvic acid into lactic acid under anaerobic conditions. Five isoforms of LDH have been identified in five different combinations of peptide subunits. In animal experiments, upregulation of LDH can ensure effective glycolytic metabolism in hypoxic tumor cells and reduce the dependence of the tumor cells on oxygen and lead to tumor progression [8–9]. In patients with HCC receiving TACE treatment, the serum LDH level also fluctuated to varying degrees, suggesting that LDH might be related to the efficacy of TACE. Therefore, this study aimed to evaluate the efficacy of LDH measurements in patients with HCC treated by TACE.

Materials and methods

Research object

A total of 52 patients receiving TACE therapy (iodide oil or drug elution microspheres) and admitted in our hospital (Huangzhou District People's Hospital, Huanggang, China) from June 2015 to December 2017 were retrospectively analyzed. The cohort consisted of 12 female and 40 male patients, with an average age of 43 years. All patients were diagnosed with primary liver cancer, and the diagnostic criteria were "Diagnosis and Treatment of Primary Liver Cancer" (2017 Edition) [10]. Patients were diagnosed through liver imaging, alpha fetoprotein markers, and clinical history; among them, 29 patients were diagnosed through liver biopsy, and those with metastatic tumors and severe complications were excluded. The pretreatment assessment was performed based on the Eastern Cooperative Oncology Group physical status, and the Child-Pugh and BCLC staging system were used to identify the stages [10]. All patients were classified as BCLB B or C. Based on the serum LDH levels before TACE, all patients were divided into the observation group (LDH of > 450 U/L) and control group (LDH of ≤ 450 U/L). Based on the changes of serum LDH level after TACE treatment, all patients were divided into two groups: LDH increased group and LDH decreased group. No significant difference in age, sex, and liver function was observed between the two groups at baseline ($P > 0.05$).

Therapeutic method

The Seldinger method was used to place the 4-Fr catheter into the proper hepatic artery or its branches through the femoral artery. Digital angiography of the artery was performed to assess the anatomical structure of the hepatic artery, in order to determine the blood supply to the liver tumor and the size and number of tumors. A microcatheter was inserted into the blood supply artery, and patients were injected with different doses of doxorubicin, tegafur, and iodide suspension based on the patient's condition. Then, the gelatin sponge particles were injected into the arterial embolism until the tumor had no contrast. The amount of contrast medium and lipiodol in the suspension was adjusted according to the size of the tumor. Routine liver protection examination was performed before and after treatment. If chronic hepatitis B existed, antiviral therapy was added.

Determination of serum LDH level

Fasting venous blood was collected and serum LDH levels were recorded before (within 1 month) and after treatment (after 1 month). According to the method of International Federation of Clinical Chemistry and Laboratory Medicine, the serum LDH level was determined using the Backman DXC800 analyzer. Patients were divided into two groups according to the LDH serum concentration recorded before TACE: the control (LDH of ≤ 450 U/L) and observation group (LDH of > 450 U/L). The cut-off value of the LDH serum level was set to 450 U/L based on the upper limit of normal LDH values seen in blood samples at our hospital. Patients were classified according to the type of change (increase or decrease) in serum LDH level before and after treatment. The ethics committee of our hospital approved the analysis. The patients also agreed to the anonymous storage of their clinical information in the hospital database and its use for research.

Follow-up and evaluation criteria

Patients who received TACE treatment were followed up for 24 months. All patients received no less than 1 images of liver function test, liver MRI or CT after TACE to evaluate the efficacy, followed by telephone follow-up. The radiologist evaluated the film, and a senior professional doctor assessed the quality of treatment.

The curative effect of the modified solid tumor (mRECIST) is considered the standard of treatment efficacy. It is divided into complete remission (CR), partial remission (PR), stable state (SD), and disease progression (PD). In CR, the development of all target lesions disappeared during the arterial phase; in PR, the diameter of the target lesion is reduced to ≥ 30% during the enhancement phase of the arterial phase; in SD, tumor reduction does not satisfy PR or increase PD; and in PD, the total diameter of the target lesion increases by ≥ 20% when new lesions occur or when the arterial phase is enhanced. The overall effective rate (ORR) was defined as the ratio between the number of patients having CR and PR in different groups and the number of patients enrolled. The disease control rate (DCR) was defined as the ratio between the number of patients having CR, PR, and SD in different groups and the number of patients

enrolled.

Statistics

SPSS 17.0 analysis software (IBM, Chicago, IL, USA) was used to analyze the data. All data were expressed as mean ± standard deviation (mean ± SD) or median (range). Chi square test was used for statistical analysis of enumeration data, and the *t*-test was used for statistical analysis of measurement data. When a P value of < 0.05 was obtained, the difference between the two groups was considered to be statistically significant.

Results

General situation

All 52 patients included in the analysis were followed up. After treatment with TACE, serum LDH levels increased in 22 patients (LDH increased group) and decreased in 30 (LDH decreased group). No significant difference was observed in the baseline data between the two groups (Table 1).

Table 1 Patients' general information (*n*)

Class	Control group (n = 26)	Observation group (n = 26)
Age (year)	43 ± 6	43 ± 5
Sex (male/female)	19/7	21/5
ALT (U/L)	68.7 ± 7.3	67.4 ± 4.9
ALB (g/L)	39.1 ± 1.2	39.5 ± 1.1
Tbil (mg/dL)	1.1 ± 0.5	0.9 ± 0.6
PT (s)	71 ± 17	70 ± 19
AFP (ng/mL)	162	173
HbsAg (+)	19	21
The number of tumor bodies (≥ 3)	11	13
The tumor size (≥ 5 cm)	23	24
Vascular invasion	12	14

Note: variables are marked by *n*, mean ± standard deviation or median (range). ALT, alanine aminotransminase; ALB, serum albumin; Tbil, total bilirubin; AFP, alpha fetoprotein

The short-term effects of TACE treatment of patients

According to the LDH level before treatment, the effective rate of treatment in the groups with oberservation and control was 42.3% (n = 11) and 57.7% (n = 15), respectively; however, no statistical difference was observed between the two groups (χ^2 = 1.23, P = 0.27). The rate of disease control in the observation and control groups was 69.2% (n = 18) and 76.9% (n = 20), respectively. The statistical analysis showed that no statistical difference was observed between the two groups (χ^2 = 0.39, P = 0.53; Table 2).

Table 2 Short-term efficacy of TACE after treatment in the observation and control groups (*n*)

Group	CR	PR	SD	PD	ORR (%)	DCR (%)
Observation group	3	8	7	8	42.3	69.2
Control group	5	10	5	6	57.7	76.9

Note: CR, complete remission; PR, partial remission; SD; disease stability; PD, disease progression; ORR; treatment efficiency; DCR, disease control rate

According to the changes of LDH levels after treatment, the effective rate of treatments in LDH increased group was found to be 41% (n = 9), and ORR in the LDH decreased group was 50% (n = 14). Statistical analysis showed that no statistical difference was observed between the two groups (χ^2 = 0.42, P = 0.52). After treatment, the rate of disease control in the LDH increased group was 63.6% (n = 14), and the DCR in the LDH decreased group was 80% (n = 24). The statistical analysis showed that no statistical difference was observed between the two groups (χ^2 = 1.73, P = 0.19; Table 3).

Table 3 Short-term efficacy of TACE after treatment in the LDH increased and decreased groups (*n*)

Group	CR	PR	SD	PD	ORR (%)	DCR (%)
Increased group	2	7	5	6	41	63.6
Decreased group	5	9	10	6	50	80.0

Note: CR, complete remission; PR, partial remission; SD; disease stability; PD, disease progression; ORR; treatment efficiency; DCR, disease control rate

The long-term effects of TACE treatment of patients

According to the pretreatment LDH level, the 1-year survival rate of the observation and control groups was 53.8% (n = 14) and 84.6% (n = 22), respectively. The statistical analysis showed that the two groups had significant statistical differences (χ^2 = 5.78, P = 0.016 < 0.05). The 2-year survival rate of the observation and control groups was 30.8% (n = 8) and 46.2% (n = 12). The statistical analysis showed that the two groups had no statistical difference (χ^2 = 1.3, P = 0.25).

Discussion

TACE is one of the commonly used methods for the treatment of HCC. However, after TACE treatment, approximately 30%–50% of patients develop extensive tumor necrosis [11]. Methods for evaluating the curative effect of TACE early, adjusting the treatment in time, and ensuring that patients receive the benefit of treatment have become controversial research topics with respect to the use of TACE treatment for liver cancer.

LDH detection is economical and convenient and can be used as a metabolic marker in patients with cancer. Combined with the patients' imaging results, it can also help determine the tumor activity and disease changes [12]. The change in LDH activity has a suggestive effect on hypoxia and anaerobic glycolysis in the tissues. Hypoxia can activate hypoxia-inducible factor (HIF), whereas HIF can promote the expression of LDH. LDH is widely distributed in the body tissues, and its high activity may suggest tissue damage [13]. Abnormal anaerobic metabolism exists in the tumor tissues and cells. Serum LDH activity and level are often increased due to tissue and cell necrosis. Studies have reported that LDH levels in patients with tumors can reach up to six times the normal level [14–15]. In lymphoma, LDH has become an important factor in determining the efficacy and prognosis. Therefore, the predictive value of LDH should be investigated to determine the efficacy of TACE in hepatocellular carcinoma.

This article reviewed the clinical value of serum LDH measurements in evaluating the efficacy of transcatheter arterial chemoembolization for primary liver cancer. In this study, patients with advanced primary liver cancer, treated by transcatheter arterial chemoembolization, were placed in either the control group (serum LDH of ≤ 450 U/L before TACE) or the observation group (serum LDH of > 450 U/L before TACE). The related clinical data were collected and analyzed, and the short-term curative effect and survival rate between the two groups were compared. The results of this retrospective study showed that compared with the control group (with LDH of ≤ 450 U/L), the treatment efficacy, rate of disease control, and survival rate were lower in the observation group, and the 1-year survival rate was significantly lower than that in the control group. After the treatment with TACE, patients were divided into 2 groups based on the type of change in LDH levels. The efficacy rate and disease control rate of LDH increased group were lower than those of LDH decreased group. These findings are consistent with previously published studies on LDH evaluating the efficacy of TACE in the treatment of HCC.

This article has the following shortcomings. First, because TACE chemoembolic drugs do not establish a standard anticancer regimen, and the content and composition of anticancer drugs used in TACE are different. Second, this was a retrospective study and a certain selection bias may have occurred. Third, although free detection of LDH and other means to ensure that patients detect LDH in the same time, but there are still some differences in sampling and testing time. Despite these shortcomings, the results are reliable.

Based on the results of this analysis, serum LDH level can become a significant prognostic factor in patients with HCC. The best individualized treatment strategy is economic and effective for patients, who are better guided by stratification in the follow-up clinical trials. In the future, large-sample comparative studies are needed to further verify the results of this study.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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ORIGINAL ARTICLE

Expression and clinical significance of serum lipoprotein (a) in patients with gastric cancer

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Abstract

Objective This study aimed to investigate the expression and clinical significance of serum lipoprotein (a) [LP (a)] in patients with gastric cancer.

Methods Two hundred and twenty-two patients with gastric cancer (gastric cancer group) were selected from 2015 to 2017 [mean age (58.40 ± 10.40) years], as were 101 healthy persons [normal age group, mean age (58.18 ± 11.42) years]. Fasting blood samples were collected and evaluated by immunoturbidimetry with a biochemical analyzer. LP (a) concentration was observed and its difference was compared.

Results There was no significant correlation between LP (a) and tumor stage ($P > 0.05$). Compared with the control group, the level of LP (a) in the male gastric cancer group was significantly higher than that in the control group ($P < 0.05$). In the subgroup analysis, the level of LP (a) and abnormal rate showed an increasing trend among patients with stages I–IV gastric cancer. The level of LP (a) in poorly differentiated gastric cancer patients was higher than that in the high middle differentiation group ($P < 0.05$). There was no significant difference in LP (a) levels among patients with different pathological types of gastric cancer ($P > 0.05$).

Conclusion LP (a) was correlated with the occurrence, development and differentiation of gastric cancer, but not with the pathological classification of gastric cancer. Serum LP (a) concentration may be used as an indicator for the staging and prognosis of gastric cancer, but the specific underlying mechanism remains to be further studied.

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Key words: lipoprotein (a) [LP (a)]; gastric cancer; staging; differentiation; pathological typing

In February 2017, the National Cancer Center released the latest cancer data for China, with about 10 000 people diagnosed with cancer every day. Gastric cancer is one of the most common malignant tumors. The incidence of gastric cancer is increasing year by year, ranking first in the incidence of gastrointestinal malignant tumors, and the mortality rate ranks second in the total mortality rate of cancer. Early detection and treatment of gastric cancer is key. The expression and clinical significance of lipoprotein (a) [LP (a)] in gastric cancer patients are discussed in this paper.

LP (a), discovered and named by the Norwegian geneticist Kåre Berg in 1963, is a complex and independent plasma lipoprotein. Apolipoprotein (a) (apo A) binds to low-density lipoprotein (LDL) via disulfide bonds, but its physical and chemical properties are essentially different from LDL [1]. It is relatively stable in individual expression and is not subject to dietary habits and geographical differences among the same races. However, LP (a) levels are largely dependent on genes, so there may be great differences between different races. Conventional studies have shown that [2–4] LP (a) increases the risk of cardiovascular disease because of its thrombotic and atherosclerotic properties. Interestingly, serum LP (a) levels were negatively correlated with levels of vascular endothelial growth factor and coronary collateral circulation development in patients with coronary heart disease. In recent years, many oncology experts [5–6] have focused on the possible role of LP (a) in tumor angiogenesis and development, and found that LP (a) has a significant impact on tumor expansion and metastasis. A small sample of data has shown that increased concentration of LP (a) increases the risk of certain cancers and affects their staging (such as breast cancer and acute myeloid leukemia). Up till now, no studies have discussed the relationship between LP (a)

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levels and the occurrence, development, staging and differentiation of gastric cancer. The aim of this study was to investigate the expression and clinical significance of LP (a) level in gastric cancer patients [7–13].

Data and methods

General information

Two hundred and twenty-two patients, 152 male and 70 female, who were diagnosed as primary gastric cancer by histopathology, aged 18–75 years, Eastern Cooperative Oncology Group (ECOG) performance status 1–2, and of Han nationality, were retrospectively enrolled in our hospital (Affiliated Hospital of Qingdao University, China) from 2015 to 2017. The inclusion criteria were body mass index (BMI) 17.9–23.9 kg/m^2, no clinical evidence of vascular disease (such as coronary artery disease, stroke, peripheral artery disease), no active inflammatory disease, and no history of drug use affecting lipid metabolism and coagulation function within 3 months. The exclusion criteria were abnormal lipid metabolism, BMI < 17.9 kg/m^2 or > 23.9 kg/m^2; clinically significant vascular disease (coronary heart disease, stroke, peripheral artery disease); abnormal liver function (transaminase and/or gamma-glutamyltransferase increased 2.5 times the upper limit of normal or clinical jaundice; abnormal renal function (creatinine or urea nitrogen concentration increased 1.5 times the upper limit of normal); coagulation system dysfunction (increased fibrin 2 times the upper limit of normal or D-dimer > 800 ng/mL); and application of drugs affecting lipid metabolism and coagulation function within the preceding 3 months. One hundred and one age-matched healthy persons undergoing check-up were included in the same period.

Data acquisition and evaluation criteria

Pathological analysis and grading of biopsy specimens were performed via endoscopic examination, laparoscopic resection and laparotomy. Ultrasound, computed tomography, magnetic resonance imaging, and bone scan were used to confirm the histological classification of gastric cancer, including adenocarcinoma, mucinous adenocarcinoma and signet ring cell carcinoma. All patients with carcinoma *in situ,* stage I, stage II, stage III, and stage IV were identified according to the staging system of the 2007 V1 National Comprehensive Cancer Network guidelines. Serum LP (a) concentration was measured in the fasting state by the immunoturbidimetric method.

Statistical analysis

SPSS version 22 (IBM Corp., Armonk, NY, USA) was used to analyze the data. The measurement data were skewed on the normality test, and the classified variables were expressed as frequencies and percentages. The comparison between groups was performed by analysis of variance, and rates were compared using the chi-square test. The correlation between serum LP (a) and tumor staging was evaluated using the independent samples *t*-test. Differences were statistically significant at $P < 0.05$.

Results

There was no statistically significant difference in the concentration of LP (a) between the gastric cancer group and control group ($P > 0.05$). The differences in LP (a) between the gastric cancer group and control group and between different stages are compared in Fig. 1.

The concentration of LP (a) in male patients with gastric cancer [mean: (325.56 ± 279.06) mg/L] was compared with that in the control group [mean: (169.08 ± 8.56) mg/L] ($P = 0.000$). Subgroup analysis of different stages showed that stage 0 [mean: (116.50 ± 23.39) mg/L] and stage I [mean: (127.82 ± 6.92) mg/L] had higher values than those of stage II [mean: (149.04 ± 10.53) mg/L] ($P = 0.094$); stage II was compared with stage III [mean: (405.30 ± 36.62) mg/L] ($P = 0.000$), stage III was compared with stage IV [mean: (469.07 ± 49.60) mg/L] ($P = 0.298$), and stage IV was compared with the healthy control group ($P = 0.000$). The relationship between LP (a) and the depth of tumor invasion, local lymph node metastasis, nerve invasion, vascular tumor embolism, and *HER-2* expression were also investigated. The results showed that LP (a) level was positively correlated with neurological invasion and vascular tumor thrombus channel positive, and LP (a) level was $P < 0.05$ in the comparison of positive and negative neurological invasion and vascular invasion, while the depth of tumor invasion was deep, local lymph node metastasis $P > 0.05$. The clinical characteristics of male patients with gastric cancer and male health examiners are shown in Table 1. The differences in LP (a) levels between male patients with gastric cancer and male health examiners and between different stages were compared as shown in Fig. 2.

LP (a) levels in male patients with poorly differentiated gastric cancer [mean: (342.86 ± 26.01) mg/L] were significantly higher than those in patients with high-moderately differentiated gastric cancer [mean: (237.68 ± 34.26) mg/L] ($P = 0.018$). The differences in LP (a) level between the gastric cancer groups according to different degrees of differentiation are shown in Fig. 3.

There was no significant difference in LP (a) levels among patients with gastric adenocarcinoma, mucinous adenocarcinoma and signet ring cell carcinoma ($P > 0.05$). The differences in LP (a) concentration between different pathological types of gastric cancer are shown in Fig. 4.

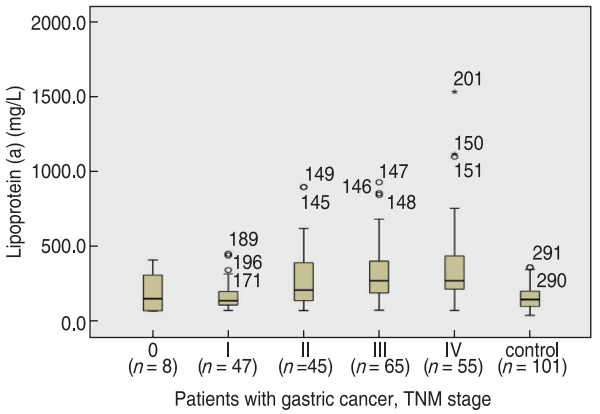

Fig. 1 Differences in lipoprotein (a) level among patients with gastric cancer according to TNM stage

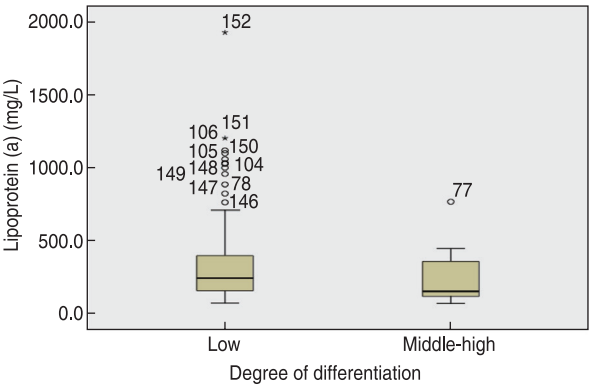

Fig. 3 Comparison according to the degree of differentiation. Note: Patients with highly differentiated and moderately differentiated gastric cancer were few and were thus combined into one group

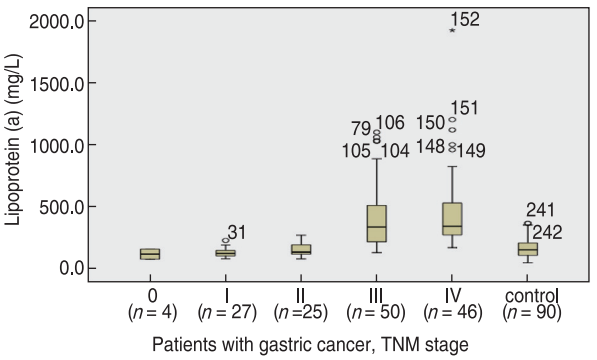

Fig. 2 Differences in lipoprotein (a) level among male patients with gastric cancer according to TNM stage

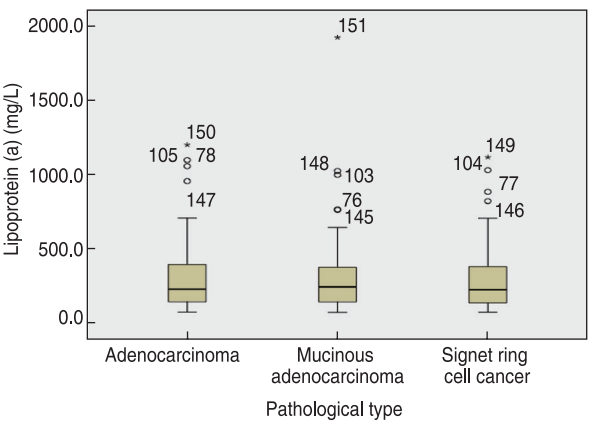

Fig. 4 Comparison of LP (a) concentration according to pathological type

Discussion

LP (a) is formed by binding to LDL through apo A disulfide bonds. It is synthesized and secreted from the liver into the blood. It is relatively stable in individual expression. It is not subject to dietary habits and geographical differences among the same race. The level of LP (a) varies greatly among different individuals and races, and the level of LP (a) is biased in the population. Estradiol-based estrogen promotes the synthesis of nucleic acids and proteins in target cells such as uterus, promotes cell growth, raises plasma α-globulin, increases plasma alpha-lipoprotein levels, and lowers LDL and cholesterol levels. Estrogen (mainly estradiol) promotes the synthesis of nucleic acids and proteins in uterine and other target cells, promotes cell growth, increases plasma

Table 1 Clinical characteristics of male gastric cancer patients and male health checkup personnel (*n*)

Variable		CIS	I	II	III	IV	Healthy people
Age (years)		64.00 ± 12.73	58.27 ± 8.48	55.22 ± 11.40	59.33 ± 10.88	58.7 ± 10.29	58.18 ± 11.42
BMI (kg/m^2)		21.59 ± 3.23	22.21 ± 1.23	22.19 ± 1.27	21.75 ± 1.49	21.45 ± 1.67	21.27 ± 1.43
Lipoprotein (a) (mg/L)		116.50 ± 23.39	127.82 ± 6.92	149.04 ± 10.53	405.30 ± 36.62	469.07 ± 49.60	169.08 ± 8.56
Degree of differentiation	Low	1	20	20	43	43	
	Middle-high	3	7	5	7	3	
Nerve invasion	+	1	2	13	42	33	
	–	3	25	12	8	13	
Vascular invasion	+	0	1	11	35	31	
	–	4	26	14	15	15	

alpha-globulin level, increases plasma alpha-lipoprotein levels, and reduces LDL and cholesterol levels. Studies have shown [14] that apo A, a component of LP (a), has 80% homology with plasminogen. LP (a) which has no fibrinolytic activity, can compete with plasminogen for the binding sites of fibrinogen monomer, inhibit the activation of plasminogen, and lead to local thrombosis and fibrinogen network. This is not only conducive to the adhesion of tumor cells to blood vessels, but also at the corresponding sites, platelet-derived growth factor can also be activated to promote the proliferation and growth of tumor cells. Other studies have shown that [15] LP (a) initiation involves a series of complex changes, such as complement activation, peptide release and chemotaxis, which play an important role in the development of tumors. In addition, some researchers [16] found apo A mRNA in cancer cell lines, so the process of cell proliferation may be due to gene mutations in the synthesis of apo A resulting in an increase in the blood level of LP (a). Some studies suggest that estrogen stimulates cells to secrete LDL receptor mRNAR. LP (a) is structurally similar to LDL. In addition to the component containing LDL (apo B 100), there is a special antigenic component apo A. LP (a) combines apo A with apo B 100 by disulfide bonds [17]. Decreased levels of estrogen can decrease plasma alpha-globulin, decrease plasma alpha-lipoprotein level, increase LDL level, and increase LP (a) level [18]. Decreased estrogen levels in postmenopausal women increase LP (a) levels, interfering with the level of LP (a) in gastric cancer patients compared with healthy people, hence the high incidence of gastric cancer among the middle-aged and elderly.

Firstly, the results of this study showed that male gastric cancer patients had significantly higher LP (a) levels and abnormal rates than normal people. Cao Chun [19] and other authors reported that serum LP (a) levels among cancer patients including lung cancer patients were significantly higher than those of normal people. It is suggested that high concentration of LP (a) may play a role in promoting tumor progression, which may be related to the fibrinolytic system and coagulation. The study also found that LP (a) levels and abnormal rates in male patients with stage I, stage II, stage III and IV gastric cancer were increasing. LP (a) levels in patients with carcinoma *in situ* were higher than those in patients with stage I and stage II disease, with small sample size bias not included in the comparison. This was consistent with the reports of Yang *et al* [14, 20–21] and other reports that the expression of lipoprotein in male lung cancer patients with stage I, II and III disease was increasing. The statistical results of this study showed that LP (a) levels were positively correlated with neurological invasion, vascular invasion, and positive and negative comparison. LP (a) may play a role in tumor angiogenesis. LP (a) contains apo A. In animal experiments [3], recombinant apo A inhibited the formation of capillary-like structures induced by basic fibroblast growth factor and tumor necrosis factor alpha. It is speculated that increased LP (a) concentration in solid tumor patients may be the regulatory mechanism of LP (a) expression under these conditions, and this may also be the case in gastric cancer. However, the specific mechanism is unclear and needs further studies to be elucidated.

The level of LP (a) in stage IV patients was higher than that in stage III patients, but the *P* value was higher than 0.05. The difference was not statistically significant. This result was contrary to that of Yang *et al* [14, 20–22] in their study of male lung cancer, but the level of LP (a) in stage IV patients was lower than that in stage III patients. In fact, the level of LP (a) in patients with stage IV disease is inconsistent. The reason may be that there are differences in the level of LP (a) between different races. LP (a) is relatively stable in individual expression, and is not subject to dietary habits and geographical differences between races, but largely depends on genes, so there may be great differences between races. The results showed that LP (a) level in poorly differentiated gastric cancer patients was higher than that in moderately-well differentiated gastric cancer patients, suggesting that the level of LP (a) might be an indicator of prognosis in gastric cancer patients, but there was no significant difference in LP (a) level between different pathological types. At present, there are no other similar or similar experimental studies, and much data need to be confirmed.

Conclusion

The aim of this study was to investigate the expression and clinical significance of serum LP (a) in patients with gastric cancer. Serum LP (a) level in patients with gastric cancer was significantly higher than that in the normal control group. The later the stage, the higher was the LP (a) level; there was also a negative correlation between LP (a) and the degree of differentiation of gastric cancer. The lower the degree of differentiation, the higher was the level of LP (a), and the correlation between LP (a) and the occurrence and development of gastric cancer. Because the experimental population selected in this experiment was mainly diagnosed and treated in recent years, it was impossible to calculate the survival period. It is not yet possible to study the relationship between LP (a) and prognosis. However, many studies have reported on the relationship between serum LP (a) levels and malignancies such as lung cancer and hematologic malignancies. The prognosis of tumors showed positive correlation. As the LP (a) level increased, the prognosis was worse. The relationship between the serum LP (a) and the prognosis of gastric cancer patients was considered as a follow-up research topic. In the future, serum LP (a) concentration

may be used as an indicator to analyze the staging and prognosis of gastric cancer, but the specific mechanism requires further research and a large amount of clinical data is needed.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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ORIGINAL ARTICLE

Clinical study of IL-18 and NANOG gene polymorphisms in prostate cancer patients*

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Abstract

Objective Recent studies have shown abnormal expression of NANOG and IL-18 to be related to cancer. However, the molecular mechanism by which IL-18 and NANOG gene polymorphisms are associated with prostate cancer is unclear. In this study, we examined whether IL-18 and *NANOG* gene polymorphisms and their interaction with prostate cancer-related risk factor are associated with the susceptibility to and clinicopathological development of prostate cancer among Chinese men.

Methods Polymorphisms in the *NANOG* and IL-18 genes were evaluated for susceptibility in 120 patients with prostate cancer. The control group consisted of 125 samples from Chinese men. Genotyping was conducted using TaqMan allelic discrimination assays. Statistical analysis was performed using SPSS software.

Results No association of *NANOG* and IL-18 gene polymorphisms and overall prostate cancer susceptibility was detected. The IL-18-607 CC genotype was significantly associated with a higher tumor grade (P = 0.025) and stage (P =0.001). The IL-18-137 GG genotype correlated with a higher tumor grade (P = 0.028) and stage (P = 0.008). The IL-18-137G allele was significantly more frequent in patients with lymph node metastasis (P = 0.035). The IL-18-607 CC genotype was associated with distant metastasis (P = 0.025). However, no significant association was observed between *NANOG* polymorphisms and any clinicopathological feature. The Cox proportional hazard model showed that tumor grade and stage grouping were independent prognostic factors in IL-18, while IL-18 polymorphism was not. Polymorphism variants in the IL-18 (IL-18-607 and IL-18-137) and *NANOG* (genotypes AC) genes might be associated with a worse prognosis of patients with prostate cancer.

Conclusion *NANOG* may be associated with the early stages of prostate cancer carcinogenesis. IL-18 and NANOG gene polymorphisms may play a major role in the growth, invasion, and metastasis of prostate cancer.

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Key words: interleukin-18; NANOG; polymorphism; prostate cancer; clinical characteristics

Embryonic stem cells are known as "cells that start the tumor" [1]. These cells possess self-renewal capacity, which enables differentiation into heterogeneous mature cells types, including tumor cells. Numerous primary non malignant and malignant tumor derived human prostate epithelial cell lines have been developed using a retroviral vector encoding the human telomerase reverse transcriptase. These cell lines exhibit characteristics of stem cells and express embryonic stem (ES) cell markers, such as *NANOG*, octamer 4 (*OCT4*), and SRY box 2(*Sox2*) [2]. The NANOG gene is located on chromosome 12 and plays a vital role in cell differentiation. Recent studies found that abnormal expression of NANOG, OCT4, and SOX2 is related to colorectal and lung cancer [3–7].

Although these factors are necessary for stem cells to acquire pluripotency, they have also been suggested to possess oncogenic potential in normal cells. However, the role of these factors in normal cells and cancer cells

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is unclear. Additional studies are needed to determine the influence of these factors on the proliferation and metastatic potential of cancer.

Recent studies showed that prostate tumor cells secrete interleukin (IL)-18 in response to interferon-γ in the tumor microenvironment and that IL-18 acts as an autocrine or paracrine factor in the tumor [8]. In some animal models, IL-18 gene transfection into tumor cells enhanced both specific and nonspecific antitumor immune responses, indicating that if the IL-18 gene is transferred into dendritic cells, it can induce highly effective antitumor immune responses [9]. These findings suggest an association between the susceptibility to cancer and IL-18 gene. We previously showed that IL-18 plays an important role in prostate cancer growth and metastasis. Additionally, our research and a recent study revealed a correlation between serum IL-18 and vascular endothelial growth factor (VEGF) levels in patients with prostate and ovarian cancer [10–11]. The IL-18 gene is located on chromosome 11q22. Two functional gene polymorphisms, -607A/C and -137G/C, are found in its promoter region. Giedraitis *et al*. analyzed the IL-18 gene promoter sequence and found a change from the C allele to A allele at position -607 and change from G to C at position-137 [12]. Our study showed that polymorphisms in the IL-18 promoter affect prostate cancer progression and prognosis. IL-18 is strongly correlated with a higher tumor grade and stage, lymph node involvement, and distant metastasis [13]. However, the molecular mechanism by which IL-18 gene polymorphisms are associated with prostate cancer is unclear.

Therefore, in this study, we recruited 245 participants, including 120 patients with prostate cancer and 125 healthy men, to determine whether IL-18 and *NANOG* gene polymorphisms and their interactions with prostate cancer-related risk factors are associated with the susceptibility to and clinicopathological development of prostate cancer among the Chinese men.

Materials and methods

Patients

A total of 120 patients with prostate cancer who underwent radical prostatectomy between 2005 and 2011 in the Department of Urology, The Affiliated Hospital of Nantong University (China) were investigated. To eliminate the influence of other diseases, we excluded patients with infectious diseases and diabetes mellitus. No patient with prostate cancer was subjected to chemotherapy, hormonal therapy, or radiotherapy before surgery. The patients including non-metastases in 80 cases and metastases in 40 cases were aged 58–85 years (mean age, 70.43 ± 11.14 years). The tumor stage was assigned according to the Whitmore-Jewett stage. The tumor was assigned according to the Gleason score. Patients were divided into groups with low (≤ 6) and high (> 6) Gleason scores. The patients and tumor characteristics are listed in Table 1. Bone metastases were assessed by bone X-ray and bone scanning. Extraosseous metastases were assessed by surgical biopsy. Recurrence was defined as a significant elevation in PSA and/or new symptoms because of local tumor recurrence. The control group contained 125 healthy volunteers (mean age 70.70 ± 9.41 years) who visited the general health check-up division at The Affiliated Hospital of Nantong University. Selection criteria for controls were no evidence of any personal or family history of cancer or other serious illness. The mean age of the control group was 69.4 years. The median follow-up time was 16 months (range: 6–38 months) after surgery, and the study was performed with the approval of the ethics committee of the Chinese Human Genome.

Table 1 Clinicopathological characteristics of prostate cancer patients (*n*)

Characteristic	No. of patients	%
Age (years)		
Mean	70.43 ± 11.14	
Range	58–85	
Tumor stage		
Lymph node metastasis		
A	5	4.2
B	67	55.8
C	10	8.3
D	38	31.7
Lymph node metastasis		
Negative	70	58.3
Positive	50	41.7
Metastasis		
Negative	80	66.7
Positive	40	33.3
Grade		
≤ 6	63	52.5
> 6	57	47.5

DNA extraction

Genomic DNA was extracted from EDTA-anticoagulated peripheral blood leukocytes by the salting-out method. Briefly, 5 mL of blood was mixed with Triton lysis buffer (0.32 M sucrose, 1% Triton_100, 5 mM $MgCl_2$, H_2O, and 10 mM Tris-HCl, pH 7.5). Leucocytes were centrifuged and washed with H_2O. The pellet was incubated with proteinase K at 56°C and subsequently salted out at 48°C using a saturated NaCl solution. Precipitated proteins were removed by centrifugation. The DNA in the supernatant fluid was dissolved in 300 mL H_2O.

IL-18 genotype

Genotyping of the two IL-18 polymorphisms was performed using predesigned TaqMan single-nucleotide polymorphism (SNP) Genotyping Assays (Applied Biosystems, USA). The Assays-on-Demand SNP genotyping kit was used for polymerase chain reaction (Applied Biosystems). Single-nucleotide polymorphism amplification assays were performed according to the manufacturer's instructions. Briefly, a 25-μL reaction solution containing 10 ng of DNA was mixed with 12.5 μL of 2 × TaqMan Universal PCR Mix (Applied Biosystems) and 1.25 μL of predeveloped assay reagent from the SNP genotyping product (Applied Biosystems) containing two primers and two MGB TaqMan probes. Reaction conditions consisted of preincubation at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. Amplifications and analysis were performed in an ABI Prism 7500 Sequence Detection System (Applied Biosystems) using SDS 1.4 software for allelic discrimination (Applied Biosystems). The following SNPs were typed: IL-18-137 G/C (rs187238) and IL-18-607 A/C (rs1946518).

NANOG genotype

Tag SNPs were selected using Haploview software 4.2 (Mark Daly's laboratory of Broad Institute, USA) [14] based on the GIH population data of HapMap (HapMap Data Rel 27 Phase II + III, Feb 09, on NCBI B36 assembly, dbSNP b126). Tag SNPs that captured all known common SNPs (with minor allele frequencies of > 0.1) in *NANOG*, with a pairwise correlation $r^2 > 0.8$, were selected. Tagger SNP rs1105786 (minor allele frequency, MAF =0.376) was found to represent known SNPs in haplotype blocks 2 in *NANOG* in the Chinese population.

Statistical analysis

SNP allele frequencies were tested for departure from Hardy-Weinberg equilibrium before analysis. Genotype frequencies were compared using the Pearson χ^2 test for 2 × 2 tables or Fisher's exact test when the expected frequency value was < 5. Patients were classified in a dichotomous manner for each of the following clinical parameters: tumor diameter, nuclear grade, tumor stage, lymph node metastasis, distant metastasis, stage grouping, and survival. The distribution of polymorphisms for each parameter was studied by analyzing the genotype group and allele frequency. Odds ratios (ORs) and significance (*P* values) were also calculated. The influence of each variable on survival was assessed using the Cox proportional hazard model. Values of $P < 0.05$ were considered significant. SPSS software version 11.5 was used for all statistical analyses (SPSS, Inc., USA).

Table 2 Associations of IL-18 genotypes with tumor risk (*n*, %)

IL-18 Polymorphisms	PC patients	Healthy controls	OR (95% CI)	*P*
-137 C/G Genotype				
CC	6 (5.0)	10 (8.0)	1.55 (0.45–4.05)	0.522
CG	47 (39.2)	40 (32.0)	1.00 (Reference)	
GG	67 (55.8)	75 (60.0)	0.78 (0.47–1.15)	0.725
Allele				
C	56 (23.3)	75 (30.0)	1.00 (Reference)	
G	184 (76.7)	175 (70.0)	1.45 (0.75–1.87)	0.072
-607 A/C Genotype				
AA	13 (10.8)	10 (8.0)	1.22 (0.56–1.96)	0.657
AC	61 (50.8)	65 (52.0)	1.00 (Reference)	
CC	46 (38.3)	50 (40.0)	0.787 (0.61–1.33)	0.322
Allele				
A	114 (47.5)	110 (44.0)	1.00 (Reference)	
C	126 (52.5)	115 (46.0)	1.36 (0.81–1.69)	0.381

Results

Correlation of IL-18 gene polymorphisms with prostate cancer clinicopathological characteristics

This case-control study revealed similar frequencies in the distribution of IL-18-137 and -607 polymorphisms between healthy controls and patients with prostate cancer. Table 2 presents the genotype distributions and statistical analysis results. The observed genotype frequencies were in accordance with Hardy-Weinberg equilibrium. The association of the IL-18 genotypes with tumor grade and stage were shown in Table 3. Genotype GG of IL-18-137 was associated with a more advanced cancer stage (OR: 2.61; 95% CI: 1.15–5.37; $P = 0.008$) and higher tumor grade (OR: 3.32; 95% CI: 1.16-8.17; $P = 0.028$). The IL-18-137 G allele was correlated with a more advanced stage (OR: 1.73; 95% CI: 1.04–3.42; $P = 0.027$) and higher tumor grade (OR: 2.13; 95% CI: 0.98–4.12; $P = 0.040$). The IL-18-607 CC genotype was significantly more frequent in patients with more advanced cancer stages (OR: 3.82; 95% CI: 1.67–7.67; $P = 0.001$) and higher tumor grade (OR: 3.11; 95% CI: 1.05–10.25; $P = 0.025$). The IL-18-607 C allele was associated with a more advanced cancer stage (OR: 2.37; 95% CI: 1.28–3.73; $P = 0.001$). The associations of IL-18 genotypes with lymph node metastasis and distant metastasis were shown in Table 4. The IL-18-137G allele was significantly more frequent in patients with lymph node metastasis (OR: 3.82; 95% CI:0.95–15.17; $P = 0.035$). The IL-18-607 CC genotype was associated with distant metastasis (OR: 2.71; 95% CI: 1.25–6.14; $P = 0.025$).

Table 3 Associations of IL-18 genotypes with tumor stage, and grade (*n*, %)

IL-18 polymorphisms	Tumor stage		Odds ratio (95% CI)	*P*	Tumor grade Odds		Ratio (95% CI)	*P*
	A–B	C–D			≤ 6	> 6		
-137 C/G Genotype								
CC	4 (5.6)	2 (4.2)	1.25 (0.21–7.81)	0.625	3 (4.7)	3 (5.3)	1.36 (0.15–16.27)	0.620
CG	35 (48.6)	14 (29.2)	1.00 (Reference)		35 (55.6)	11 (193)	1.00 (Reference)	
GG	33 (45.8)	32 (66.7)	2.61 (1.15–5.37)	0.008	25 (39.7)	43 (75.4)	3.32 (1.16–8.17)	0.028
Allele								
C	35 (29.2)	32 (24.8)	1.00 (Reference)		55 (42.3)	32 (26.2)	1.00 (Reference)	
G	85 (70.8)	97 (75.2)	1.73 (1.04–3.42)	0.027	75 (57.7)	90 (73.7)	2.13 (0.98–4.12)	0.040
-607 A/C Genotype								
AA	8 (11.1)	3 (6.2)	0.83 (0.26–2.37)	0.617	5 (7.9)	4 (7.0)	1.17 (0.27–5.05)	0.782
AC	44 (61.1)	21 (43.8)	1.00 (Reference)		44 (69.8)	29 (50.9)	1.00 (Reference)	
CC	20 (27.8)	24 (50.0)	3.82 (1.67–7.67)	0.001	14 (22.2)	24 (42.1)	3.11 (1.05–10.25)	0.025
Allele								
A	57 (43.2)	45 (35.4)	1.00 (Reference)		35 (26.9)	23 (17.7)	1.00 (Reference)	
C	75 (56.8)	82 (64.6)	2.37 (1.28–3.73)	0.001	95 (73.1)	107 (82.3)	1.78 (0.87–4.52)	0.153

Table 4 Associations of IL-18 genotypes with Lymph node metastasis, metastasis and Stage grouping (*n*, %)

IL-18 polymorphisms	Lymph nodemetastasis		OR (95% CI)	*P*	Metastasis		OR (95% CI)	*P*
	A–B	C–D			≤ 6	> 6		
-137 C/G Genotype								
CC	2 (2.9)	1 (2.0)	0.84 (0.88–1.12)	0.672	2 (2.5)	1 (2.5)	2.81 (0.32–13.27)	0.427
CG	30 (42.9)	23 (46.0)	1.00 (Reference)		35 (43.8)	7 (17.5)	1.00 (Reference)	
GG	38 (54.2)	26 (52.0)	1.79 (0.33–8.35)	0.436	43 (53.7)	32 (80.01)	1.98 (0.92–5.17)	0.163
Allele								
C	65 (43.3)	5 (16.7)	1.00 (Reference)		42 (25.1)	11 (16.2)	1.00 (Reference)	
G	85 (56.7)	25 (83.3)	3.82 (0.95–15.17)	0.035	125 (74.9)	57 (83.8)	1.57 (0.82–3.50)	0.317
-607 A/C Genotype								
AA	3 (4.3)	2 (4.0)	0.87 (0.89–1.03)	0.343	5 (6.3)	2 (5.0)	2.47 (0.67–7.15)	0.168
AC	40 (57.1)	16 (32.0)	1.00 (Reference)		47 (58.7)	15 (37.5)	1.00 (Reference)	
CC	27 (38.6)	32 (64.0)	2.62 (0.68–9.67)	0.152	28 (35.0)	23 (57.5)	2.71 (1.25–6.14)	0.025
Allele								
A	60 (46.2)	5 (18.5)	1.00 (Reference)		61 (34.7)	21 (28.8)	1.00 (Reference)	
C	70 (53.8)	22 (81.5)	2.98 (0.89–8.93)	0.057	115 (65.3)	52 (71.2)	1.45 (0.83–3.45)	0.237

Table 5 Associations of Nanog genotypes with tumor risk (*n*, %)

Nanog polymorphisms	PC patients	Healthy controls	OR (95% CI)	*P*
Genotype				
TT	14 (11.7)	10 (8.0)	1.31 (0.64–1.75)	0.658
CT	60 (50.0)	65 (52.0)	1.00 (Reference)	
CC	46 (38.3)	50 (40.0)	0.897 (0.75–1.52)	0.402
Allele				
T	120 (47.6)	110 (44.0)	1.00 (Reference)	
C	132 (52.4)	115 (46.0)	1.16 (0.87–1.59)	0.301

Correlation of NANOG gene polymorphisms with prostate cancer clinicopathological characteristics

The observed genotype frequencies of the *NANOG* gene polymorphisms studied in healthy controls were in accordance with Hardy–Weinberg equilibrium (Table 5). No significant differences were observed in the frequency distribution of *NANOG* rs11055786 polymorphisms between patients with prostate cancer and healthy controls, both at the genotypic and allelic levels (Table 5). We performed binary logistic regression analysis to evaluate the correlations between *NANOG*

Table 6 Correlation of Nanog gene polymorphisms with prostate cancer clinicopathological characteristics

Nanog	TT	CT + CC	OR (95%CI)	*P*	T	C	OR (95%CI)	*P*
Clincal stage								
A + B	41 (64.1)	31 (55.4)	1 (Reference)		67 (55.8)	79 (65.8)	1 (Reference)	
C + D	23 (35.9)	25 (44.6)	1.70 (0.82–3.52)	0.28	53 (44.2)	41 (34.2)	1.08 (0.72–2.15)	0.65
Grade								
≤ 6	35 (59.3)	38 (53.5)	1 (Reference)		62 (51.7)	67 (55.8)	1 (Reference)	
> 6	24 (40.7)	33 (46.5)	0.82 (0.40–1.72)	0.72	58 (48.3)	53 (44.2)	0.65 (0.49–1.24)	0.37
Lymphy node								
Negative	24 (61.5)	46 (56.8)	1 (Reference)		65 (54.2)	70 (58.3)	1 (Reference)	
Positive	15 (38.5)	35 (43.2)	6.08 (1.82–20.12)	0.24	55 (45.8)	50 (41.7)	1.26 (0.65–2.27)	0.34
Metastasis								
Negative	38 (67.9)	42 (65.6)	1 (Reference)		83 (69.2)	75 (62.5)	1 (Reference)	
Positive	18 (32.1)	22 (34.4)	1.81 (0.86–3.72)	0.39	37 (30.8)	45 (37.5)	0.56 (0.35–1.21)	0.18

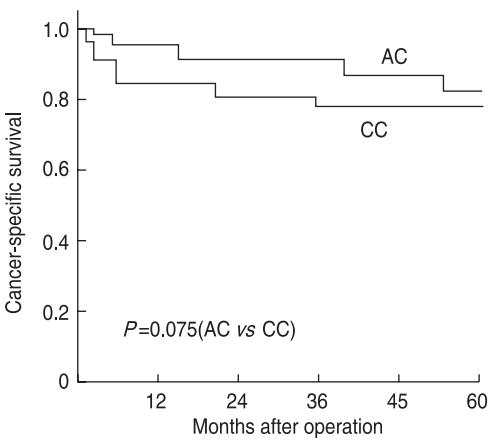

Fig. 1 Kaplan-Meier overall survival estimate according to IL-18-607 polymorphism. Differences between curves were evaluated by the Log-rank test.

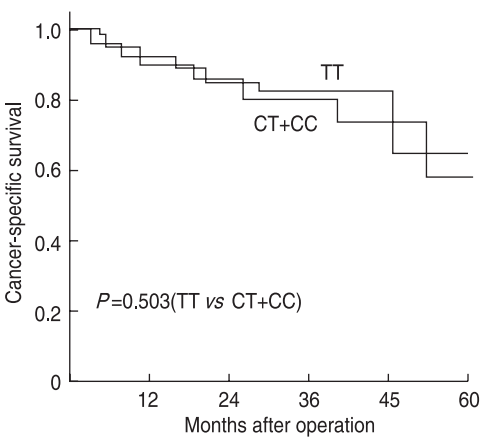

Fig. 2 Kaplan-Meier overall survival estimate according to Nanog polymorphism. Differences between curves were evaluated by the Log-rank test.

gene polymorphisms and clinicopathological features in 120 patients. However, no significant association between the *NANOG* rs1105786 polymorphism and any clinicopathological features was detected (Table 6).

IL-18 and Nanog polymorphisms in cancer survival

Thirty-four patients died of cancer-related causes during the follow-up period. Kaplan-Meier curves were calculated for cancer-specific survival by IL-18-607 genotype (AC and CC) and Nanog genotype (TT and CT + CC) (Fig. 1 and Fig. 2). Patients with the IL-18-607 genotype AC genotype showed more favorable cancer-specific survival than those with the CC genotype (P = 0.076; Log-rank test). However, the NANOG genotypes TT and CT + CC were not significantly correlated with cancer survival (P = 0.503; Log-rank test). The Cox proportional hazard model revealed that tumor grade and stage grouping were independent prognosis factors (Table 7). However, IL-18 polymorphisms, at least in this series of patients, did not serve as independent prognosis factors.

Discussion

CSCs are important in carcinogenesis and treatment resistance and may lead to metastasis. Pluripotency associated transcription factors, including *NANOG*, *Sox2*, and *OCT4*, are known to regulate cellular identity in embryonic stem cells [15] and were recently identified in

Table 7 Multivariate analysis of overall survival in prostate cancer patients

Variable	B	SE	Wald	*df*	*P*	Exp (B)
Tumor grade	1.433	0.701	5.253	1	0.035	3.476
Tumor Stage	1.575	0.527	15.217	1	0.002	4.612
IL18 -137	-1.673	1.132	4.076	1	0.073	0.180
IL18 -607	0.415	0.507	0.517	1	0.511	1.415

epithelial malignancies in a variety of tissues [16], including prostate cancer [17].

NANOG is known to control the differentiation of embryonic stem cells and plays a role in maintaining the self-repopulating ability [18]. A systematic study using animal models and *in vitro* cell systems demonstrated the key function of *NANOG* in human tumor development [19]. A recent study showed that the transforming growth factor β pathway is involved in regulating *NANOG* gene expression via binding to the *NANOG* proximal promoter [20]. Cultured human prostate cancer cells, prostate cancer xenografts, and primary prostate cancer cells express a functional variant of NANOG, *NANOG* mRNA, in cancer cells [19].

Cytokine IL-18 is known to play a critical role in the development and progression of tumors including prostate cancer. Our results revealed a strong association between increased expression of IL-18 and poor outcomes of patients with prostate cancer. Experimental studies demonstrated that IL-18 promote tumorigenesis, angiogenesis, and metastasis [21–22]. IL-18 is also known to induce multi-drug resistance to cancer cell lines [23]. Moreover, emerging evidence suggests that IL-18 has an important role in cancer stem cell phenotype and function. Additionally, IL-18 was found to enhance tumorigenicity in glioblastoma, which is consistent with the increased capacity of cancer stem cell self-renewal [24–25]. Consistent with these findings, our results showed that IL-18 might directly regulate the self-renewal capacity of cancer stem cells; however, the exact role of IL-8 in regulating CSC characteristics is not fully understood.

In the present study, we found no association between IL-18 and Nanog polymorphisms and a higher risk of prostate cancer. However, as in other studies [26–27], these polymorphisms were correlated with more advanced cancer stages. *IL-18* promoter polymorphisms have been associated with other cancers, including prostate and colorectal carcinomas [28–29], although the authors found no association between IL-18 and Nanog polymorphisms with cancer risk [30–31]. Our findings support the recent suggestion that the pleiotropic cytokine IL-18 has both anti-cancerous and pro-cancerous activities [32]. In fact, IL-18 activities are influenced by the tumor microenvironment. Thus, IL-18 can exert its antitumor activity by augmenting interferon-γ production, particularly in the presence of IL-12 [32]. However, recent data also suggested the pro-cancerous activity of this multifunctional cytokine under certain conditions depending on the tumor immune response at different tumor sites and probable genetic background [33]. In our patient group with prostate cancer, IL-18 and *NANOG* polymorphisms did not appear to be associated with prostate cancer susceptibility. This discrepancy may be attributable to the different genetic backgrounds and different environmental factors, such as the different carcinogens that initiate different cancers and different carcinogen exposures in the populations. Additionally, study design factors such as nonrandom sampling and the limited sample size should be considered. There also may have been selection bias in this hospital based, case-control study. Finally, we cannot exclude that the possibility that the association depends on a gene in linkage disequilibrium with the IL-18 gene or on the effect of IL-18 on another peptide. However, once the tumor appears, the highly productive IL-18 polymorphism promotes progression to a more advanced tumor grade, stage, etc. These results may be explained by the fact that IL-18 induces the production of angiogenic and growth factors [34–36].

We found that a genotype related to higher production of IL-18 is associated with a higher grade and stage of the tumor. IL-18 activates hypoxia-inducible growth factor [34] and vascular endothelial growth factor [35], and can activate angiogenesis in tumor nests [32]. Therefore, *IL-18* polymorphisms that increase its production would increase angiogenesis and provide adequate nutrients to transformed cells, promoting a more advanced stage. IL-18 is also correlated with disease progression. High-production polymorphisms in *IL-18* are associated with the dedifferentiation of tumor cells, leading to a more advanced tumor grade and stage grouping. Elevated IL-18 expression was found to correlate with the malignancy of skin cancers [37] and progression of breast cancer [26]. Therefore, IL-18 can directly promote proliferation by regulating proliferation stimulators. However, our findings revealed no association between prostate cancer risk and clinicopathological features. A recent study reported positive correlations between NANOG and oral cancer stem-like cells and high-grade oral squamous cell carcinoma [3]. Experimental evidence has also shown that knockdown of *NANOG* mRNA in cancer cells inhibits tumorigenesis and clonogenic growth of breast, colon, prostate, and gastrointestinal cancer cell lines [38–40]. Bioinformatic analysis showed that the rs1105786 polymorphism along with the polymorphisms in prostate cancer affected the splicing mechanism, suggesting a role for *NANOG* in tumorigenesis. No previous studies have evaluated the role of genetic variants in *NANOG* polymorphisms in prostate cancer. However, in this study, no significant association in *NANOG* rs1105786 polymorphism with tumor grade and stage was observed.

IL-18 was recently implicated in the migration of cancer and human melanoma cell lines through the generation of a region of interest and mitogen-activated protein kinase pathway [41]. Proinflammatory cytokines also induce adhesion receptors of endothelial cells for cancer cell attachment [42], which is necessary for blood-borne metastasis. Our results agree with these findings.

The clinical importance of these parameters should be investigated in patients with prostate cancer, particularly in those with bone metastasis. Additionally, larger sample sizes are needed. In the present study, polymorphisms related to IL-18 production were associated with the development of metastasis and lymph node involvement. However, polymorphisms related to Nanog were not associated with lymph node and metastasis involvement. Nevertheless, metastasis is a highly complex process that may involve numerous genes. This adds further complexity to the analysis of a specific polymorphism, as each gene is likely to contribute only moderately to the risk [43]. The association between overall survival and the IL-18-607 polymorphism was also analyzed. Because the median survival (50% mortality) was not achieved, we cannot confirm or rule out the statistical influence of this variable as a prognostic factor. Although polymorphisms related to IL-18 production were strongly correlated with more advanced stages of prostate cancer, explaining the tendency for an association with death risk ($P = 0.076$), Cox analyses revealed that *IL-18* and *Nanog* polymorphisms are not independent survival factors. *IL-18-137* and *Nanog* polymorphisms did not influence the risk of death in our patients. Thus, the influence of the IL-18-607 polymorphism may be more significant than that of IL-18-137, promoting higher-risk phenotypes, as also reported in nasopharyngeal carcinoma [32].

In conclusion, this study showed that *IL-18* promoter polymorphisms affect prostate cancer progression and prognosis. IL-18 is strongly correlated with higher tumor grade and stage, lymph node involvement, and distant metastasis. NANOG may be associated with the early stages of prostate cancer carcinogenesis. However, the molecular mechanism by which IL-18 and Nanog gene polymorphisms are associated with prostate cancer is unclear. Further studies are needed to determine whether IL-18 and Nanog polymorphisms are independent risk factors or indirect markers of different genetic and environmental factors. Additional studies may be useful for developing specific therapies against tumors based on the patient genotype.

Conflict of interest

The authors confirm that there are no conflicts of interest.

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CASE REPORT

Metastatic intracranial large-cell neuroendocrine carcinoma: a study of two cases

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Abstract

Objective The occurrence of large-cell neuroendocrine carcinoma (LCNEC), a kind of neuroendocrine tumor (NET), in the cranium is extremely rare. Here we report two such cases and review the literature in order to improve the diagnosis and treatment of intracranial LCNEC.

Methods We report two cases of metastatic intracranial LCNEC. In case 1, the patient was diagnosed with lung carcinoma and underwent chemotherapy. Brain metastases were found six months later. The lung and intracranial lesions in case 2 were found at the same time.

Results Intracranial multiple-tumor resection was performed in case 1 and the patient died 2 months later. Case 2 patient underwent surgery followed by chemotherapy with etoposide and carboplatin. Six months postoperatively, a recurrence lesion was found in the left cerebellar hemisphere. The patient was treated surgically. At present, a year after the diagnosis, the patient is still alive.

Conclusion NETs of the intracranial region are extremely rare, and hence, most of our knowledge is based on lung NETs, and standard treatment strategies for intracranial NETs remain unclear. Our patients had different survival times probably due to different treatments, indicating that effective surgical resection and subsequent multi-agent chemotherapy should be administered to promote long-term survival of intracranial LCNEC patients.

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Key words: large cell neuroendocrine carcinoma; intracranial; neuroendocrine tumor; prognosis

Large-cell neuroendocrine carcinoma (LCNEC) is a kind of neuroendocrine tumor (NET), which is extremely rare in the cranium. NETs are classified as high grade, like small cell carcinoma (SCC) and LCNEC, or low grade, like carcinoid (typical carcinoid and atypical carcinoid). Out of all these, SCC is the most common. LCNEC is extremely difficult to diagnose. Histology and immunohistochemistry (ICH) are considered to be useful for pathological diagnosis. The prognosis of intracranial LCNEC is very poor, and standard treatment remains unclear due to its rarity.

Here we report two patients with intracranial LCNEC in order to improve the diagnosis and treatment of the disease.

Case 1

A 57-year-old man was admitted to our hospital with a history for paroxysmal headache and dizziness for half a month, without nausea, emesis, tic of limbs, fever, and chills. Five months before, the patient was diagnosed with lung carcinoma and was administered chemotherapy with gemcitabine and cis-platinum. Physical examination was normal. Laboratory data were within the healthy range, except for sodium (122.3 mmol/L). Cranial computerized tomography (CT) showed multiple intracranial space-occupying lesions. Cranial enhanced magnetic resonance imaging (MRI) also revealed two abnormal circular and enhanced intracranial masses with peritumoral edema; one was approximately 3.9 cm × 3.1 cm in the left

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cerebellar hemisphere, and the other was about 1 cm × 0.7 cm in the left occipital lobe, which led us to suspect that it was a metastatic carcinoma (Fig. 1). Additional imaging test, such as the color doppler ultrasound and CT of the neck, chest, abdomen, and the genitourinary system showed that no extracranial lesions.

Intracranial multiple-tumor resection was performed successfully and the patient showed good recovery. The tumor in the left cerebellar hemisphere was approximately 2 cm × 2 cm × 1 cm and was hoary red and soft, and the tumor in left occipital lobe measured 1 cm × 0.6 cm × 0.2 cm and was hoary and soft. Postoperative pathological examination indicated solid nests of large cells with eosinophilic cytoplasm and hyperchromatic nuclei and nucleoli, focal necrosis, and a high mitotic rate (Fig. 2). IHC analysis showed CD56(+), Ki-67(+ > 80%), CK7(+), CK(+) cells (Fig. 3). A pathological diagnosis of metastatic large-cell neuroendocrine carcinoma (LCNEC) was made from the lung. The patient died in October 2017.

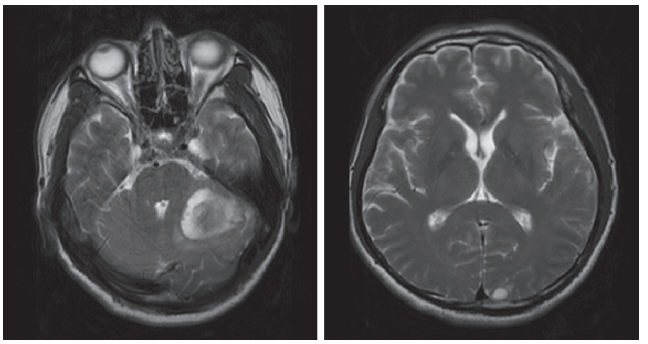

Fig. 1 MRI revealed two abnormal circular enhanced intracranial masses with peritumoral edema

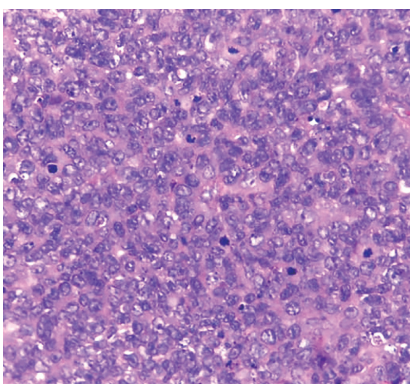

Fig. 2 Pathological examination indicated that solid nests of large cells, with eosinophilic cytoplasm, hyperchromatic nuclei and nucleoli, focal necrosis and high mitotic rate

Case 2

A 58-year-old Chinese female was admitted to our hospital with a history of headache and nausea. She had no particular family history. Neurological examination revealed parameters within healthy limits: the patient was conscious, pupil size of both the eyes was equal (about 2.5 mm), pupillary reaction to light was existent, myodynamia of all the four limbs were normal, there was normal nervous reflection of both knees, etc., and no pathological reflection of Babinski's sign was observed. The patient's laboratory data were within the healthy limits. CT showed a lesion in the upper lobe of the left lung and a lesion in the left cerebellum (Fig. 4). Pathology report from a CT-guided percutaneous lung puncture biopsy indicated poorly differentiated carcinoma. IHC analysis showed AE1/AE3(+), P40(+), CK5/6(+), CD56(+), Ki-67(+ > 80%) TTF-1(–), NapsinA(–) cells, and a diagnosis of LCNEC of the lung was made.

Intracranial tumor resection was performed successfully and the patient showed good recovery. Postoperative pathological examination indicated a poorly differentiated neuroendocrine carcinoma (Fig. 5). IHC stains were utilized with tumor cells positive for Syn, CK, and CD56 (Fig. 6). A pathological diagnosis of metastatic intracranial LCNEC was made from the lung. The patient underwent chemotherapy with etoposide and carboplatin.

Six months postoperatively, the patient presented with a 10-day history of headache and tinnitus. CT imaging demonstrated a recurrent lesion in the left cerebellar hemisphere. The patient was treated with surgery.

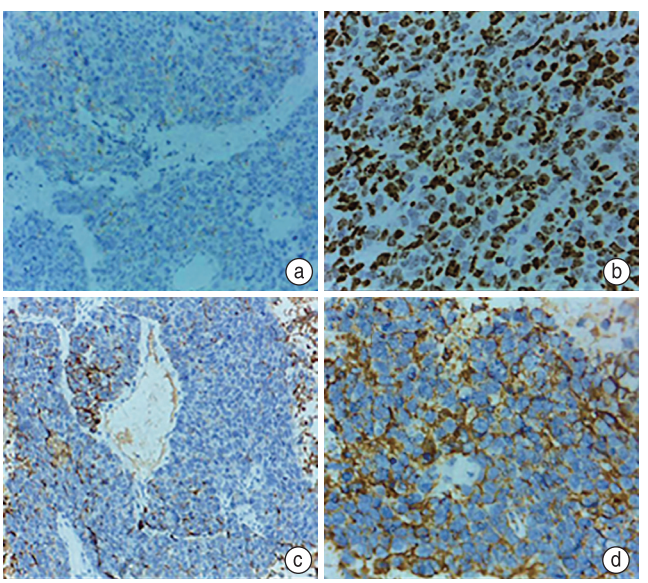

Fig. 3 Immunohistochemistry (IHC) analysis showed The neoplastic cells were positive for CD56 (a), Ki-67+ >80% (b), CK7 (c), CK (d)

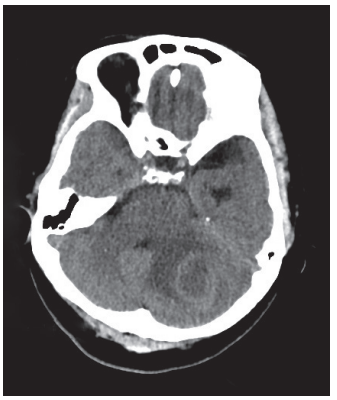

Fig. 4 CT showed a lesion in the left cerebellar region

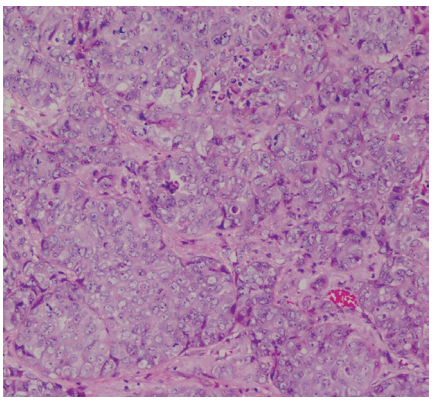

Fig. 5 Pathological examination showed poorly differentiated neuroendocrine carcinoma

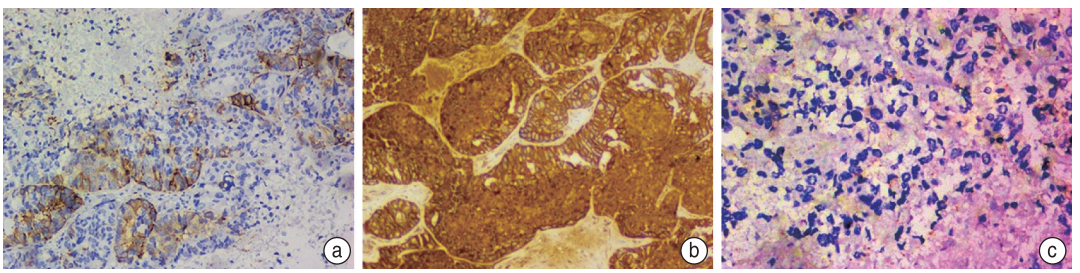

Fig. 6 IHC stains were utilized with tumor cells positive for Syn (a), CK (b), CD56 (c)

Histomorphology and IHC were confirmed intracranial LCNEC. At present, a year after the diagnosis, the patient is still alive.

Discussion

NET is a kind of heterogeneous carcinoma [1] with the very low incidence of about 5.25 cases per 100,000 [2]. It is mainly observed in the lungs, the thyroid, the jejunum, the ileum, and the pancreas [2], while intracranial NET is rarely reported. It was first described in 2005 by Peng *et al* [3]. To our knowledge, only two relevant studies on intracranial LCNEC, a kind of NET, have been reported until now [4–5].

NETs of the cranium are exceedingly rare, and consequently, most of the knowledge is based on lung NETs. In the 2015 WHO classification, lung NETs were classified as high grade, small cell carcinoma (SCC) and LCNEC, or low grade, carcinoid (typical carcinoid and atypical carcinoid). Out of all these, SCC is the most common [6]. NET tumor cell originated from neuroendocrine cells and peptidergic neurons ubiquitous in the neuroendocrine system. Travis *et al* [7] first reported lungs LCNEC and proposed that LCNEC have large and polygonal cells with a low nucleo-to-cytoplasmic ratio, coarse nuclear chromatin, and frequent nucleoli but shows more necrosis and a higher mitotic rate (>10/10 HPFs). Cytologically, LCNEC tumor cells are larger than those of SCC and have distinct instead of molded cell borders [8]. Typical carcinoids have nests of round cells and a low mitotic rate. Like LCNEC, atypical carcinoids have vesicular nuclei, prominent nucleoli, and pleomorphism and necrosis but a low mitosis rate [9].

It is extremely difficult to diagnose LCNEC before surgery. Travis *et al* [6] reported that LCNEC can only be diagnosed in a surgically resected tumor, and it should not be applied to cytology or small biopsy specimen. To confirm the neuroendocrine features, tumor cells require IHC to document neuroendocrine marker expression. Ki-67, synaptophysin, chromogranin, neuron-specific enolase, and CD56 are the most sensitive and specific [10]. Recently, a novel IHC marker, TTF-1, has been considered to be a useful marker for LCNEC [6]. Among the pulmonary tumors, TTF-1 was reported to have high positive rates in atypical carcinoid tumors (100%), SCCs (83%–100%) and LCNECs (25%–75%) [5].

In our patients, case 1 presented with paroxysmal headache and dizziness, and was diagnosed using histology and immunohistochemistry. Pathology of the tumor showed that large cells with hyperchromatic nuclei and nucleoli, focal necrosis, and high mitotic rate, all of which aided in distinguishing it from a SCC and carcinoid. IHC analysis revealed the neoplastic cells were positive for CD56, Ki-67 (> 80%), CK7, and CK.

Histomorphology and IHC of this patient were confirmed LCNEC. Case 2 presented with headache and nausea. IHC analysis showed Syn, CD56, CK7, CK, and a pathological diagnosis of metastatic intracranial LCNEC was made. It is extremely difficult to diagnose LCNEC. In order to improve the diagnosis of intracranial LCNEC, additional IHC staining of synaptophysin, chromogranin, neuron-specific enolase, CD56, and Ki-67 should be performed.

Standard treatment strategies for intracranial NETs remain unclear due to the rarity of this neoplasm [11–12]. High-grade tumors are frequently regarded as a systemic disease [13]. Cao *et al*[14] demonstrated that complete surgical treatment is warranted for symptomatic mass effect. Postoperative adjuvant chemotherapy and radiotherapy might be effective to high-grade intracranial NECs. However, Francisco *et al* [15] reported that chemotherapy is the mainstay of therapy, and surgery or radiotherapy should be considered. Van der Laan *et al* [16] reported that LCNEC, a kind of malignant cancer with poor prognosis, has a 5-year survival rate of 14.4%. Prognosis is very poor once LCNEC metastasize to the brain and most patients survive for less than 6 months [10]. Cao *et al* [14] reported that postoperative chemotherapy (YH-16 combined with temozolomide) is effective against intracranial LCNEC. The treatment of intracranial LCNEC is less reported. In our patients, case 1 underwent a complete surgery and died two months later, and case 2 underwent surgery followed by chemotherapy with etoposide and carboplatin and is still alive. There is a difference in the survival time between the two cases, which may be due to the different treatments given. This shows that effective surgical resection and subsequent multi-agent chemotherapy should be performed to promote long-term survival of intracranial LCNEC patients.

In conclusion, intracranial LCNEC is an extremely rare malignant cancer. There have been only a few reports of the disease in the literature. Here, we report a patient with intracranial LCNEC to improve the diagnosis and treatment of the disease.

Acknowledgments

The authors thank all the patients and their families for participating in this research.

Conflicts of interest

The authors declare no potential conflicts of interest.

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CASE REPORT

Olfactory schwannoma: a case report

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Abstract

Objective Intracranial schwannomas are frequently located in the VIII cranial nerve, followed by the V and VII cranial nerves, but are quite rare in the olfactory cranial nerves. Here, we report a case of an olfactory schwannoma and review the literature to improve the diagnosis and treatment of olfactory schwannoma.

Methods We report a case of olfactory schwannoma in a 51-year-old Chinese man who experienced dizziness and no other symptoms.

Results Magnetic resonance imaging (MRI) showed a neoplastic mass located on the anterior cranial base to the right of the midline and near the cribriform plate and sphenoidal plane. The lesion travelled through the cribriform plate into the nasal cavity. This mass was initially thought to be an olfactory groove meningioma. We performed a craniotomy for surgical excision, and the tumor was completely resected, and the skull base was reconstructed at the same time. There were no complications during surgery, and the patient recovered well. The histopathological diagnosis was a schwannoma.

Conclusion Olfactory schwannomas are extremely rare and similar to olfactory ensheating cell tumors, and the immunohistochemical staining of leukocyte antigen 7 (Leu7/CD57) can be used to identify them. Although the standard treatment of olfactory schwannoma remains unclear, in all reports, most patients can have excellent prognosis after an effective surgical resection.

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Key words: olfactory schwannoma; anterior skull base schwannoma; schwannoma; diagnosis; treatment

Intracranial schwannomas are frequently located in the VIII cranial nerve, followed by the V and VII cranial nerves [1–2]. Olfactory schwannoma is extremely rare. Schwannomas originate from Schwann cells and thus can grow anywhere in the nervous system that contain Schwan cells. Olfactory and optic nerves lack a Schwann cell layer, making it impossible for schwannomas to develop here [2–3]. The most common tumor developed within the anterior cranial fossa is a meningioma, with olfactory schwannoma and olfactory ensheathing cell tumor (OECT) being the rarest. Olfactory schwannoma is similar to OECT with respect to clinical and radiological characteristics, and the only way to distinguish between them is immunohistochemical staining [4].

Here, we report about a patient with an olfactory schwannoma who underwent craniotomy for surgical excision to improve its diagnosis and treatment.

Case report

A 51-year-old man with vertigo, without headache, nausea, emesis, anosmia, and seizures was referred to our hospital. Physical examination revealed a body temperature of 36.4 °C, pulse rate of 83 beats/min, and blood pressure of 122/80 mmHg. Brain MRI revealed a homogeneous, well-defined, extra-axial neoplastic lesion measuring 4.1 cm × 3.6 cm × 3.1 cm and located on the anterior cranial base to the right of the midline near the cribriform plate and sphenoidal plane, with the ipsilateral rectus gyrus compressed. The lesion extended from the cribriform plate into the nasal cavity, was hypointense on T1-weighted and hyperintense on T2-weighted images, and was heterogeneously reinforced after the application of a contrast (Fig. 1). Computed tomography (CT) scans showed a thin anterior skull base and a partly calcified

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neoplastic mass (Fig. 2). This mass was initially considered to be an olfactory groove meningioma.

The right frontopterional craniotomy approach for surgical resection was performed successfully on September 6, 2018. With an operating microscope and microsurgical technique, dissection was performed, and the tumor was completely resected and the skull base was reconstructed at the same time. There were no surgical complications, and the patient recovered well without postoperative complications such as cerebrospinal fluid leakage. Microscopically, the mass consisted of spindle-shaped cells that were partly arranged in palisades. Histological and immunohistochemical (IHC) staining was positive for S-100 protein and Leu 7 but negative for EMA and GFAP (Fig. 3). Based on histological and IHC analyses, a diagnosis of schwannoma was made.

Discussion

The most common subfrontal neoplasm is a meningioma, whereas subfrontal olfactory schwannoma is uncommon [5]. Figueiredo [6] reported an average age of diagnosis of 30.9 years among mainly male patients who have main clinical symptoms such as headache, seizures, and anosmia.

Intracranial schwannomas are frequently located in the VIII cranial nerve, followed by the V and VII cranial nerves. Optic and olfactory cranial nerves lack a Schwann cell layer; thus, the development of schwannomas at this location is impossible [2–3]. They have been labelled as subfrontal schwannoma, anterior skull base schwannoma, olfactory groove Schwannoma, olfactory schwannoma, etc. [3].

The origin of pathogenesis remains unclear. Various theories have been proposed; the developmental hypothesis suggests that pial cells transform into Schwann cells, aberrant neural crest cells in the central nervous system, and ectopic Schwann cells, and the non-development theories maintain that olfactory schwannomas originate from Schwann cells located in the perivascular nerve plexus, meningeal branches of the trigeminal nerve, anterior ethmoidal nerve, or dural nerves of the anterior cranial fossa and subarachnoid space [2–3, 6–8, 10]. Other theories include the existence of

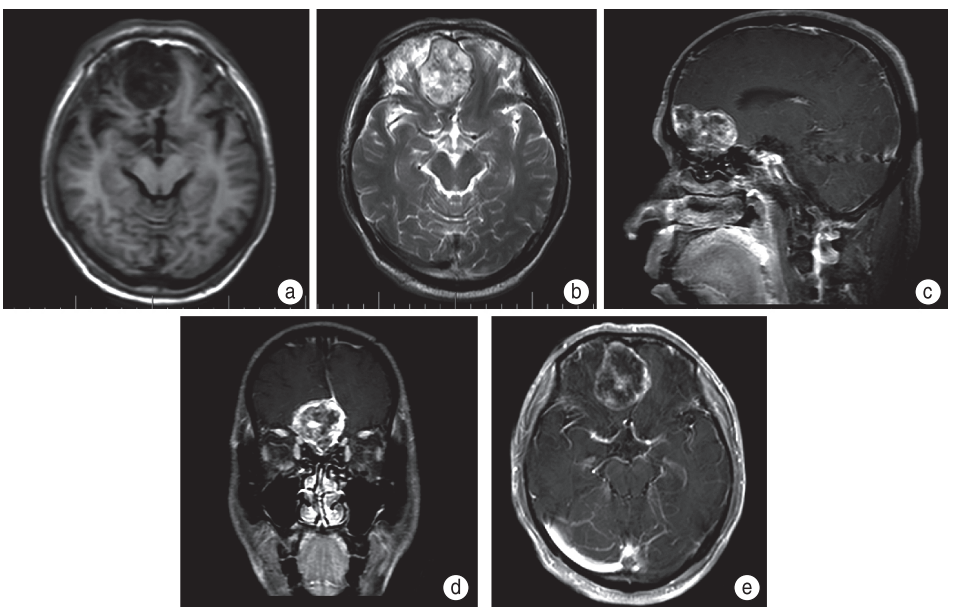

Fig. 1 MRI shows an extra-axial lesion of neoplastic, 4.1 cm x 3.6 cm x 3.1 cm, homogeneous, well defined and located on the anterior cranial base to the right of midline and throuth the cribriform plate into the nasal cavity. (a) T1 weighted is hypointense; (b) T2 weighted is hyperintense; (c–e) After application of contrast there was heterogeneous reinforcement, the margin of tumor are strongly reinforcement

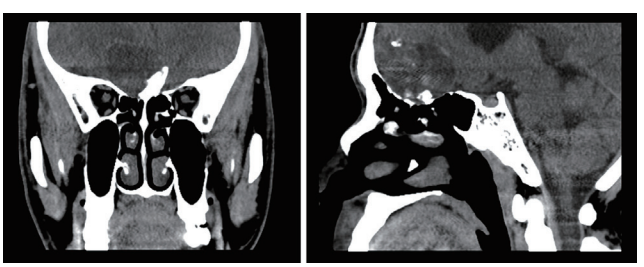

Fig. 2 The computed tomography scan reveal the anterior skull base became thin and the part of neoplastic has calcification

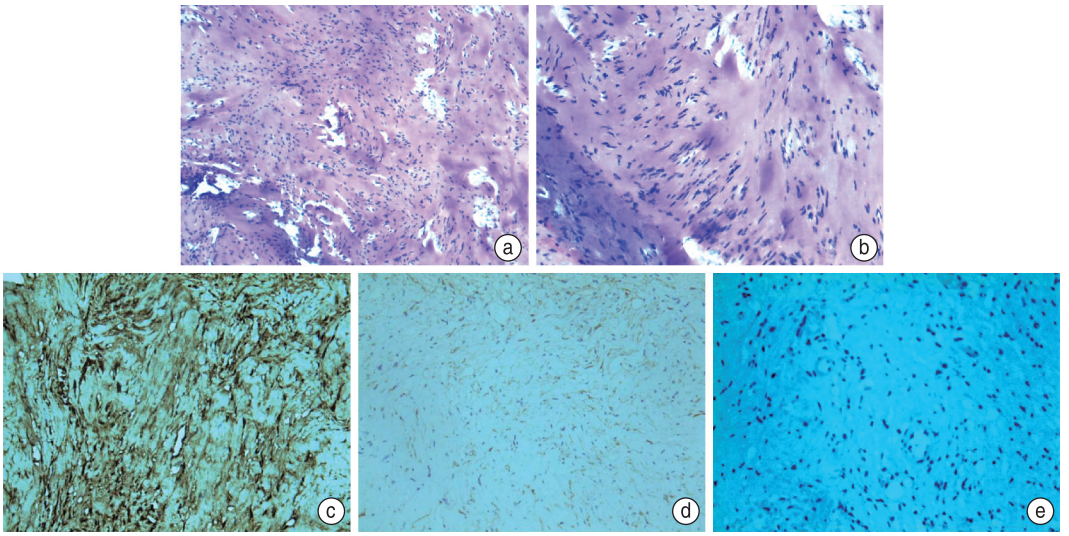

Fig. 3 Photomicrograph show the mass consisted by spindle-shaped cells and sparsely arranged, part of them arranged in palisades (HE staining, a × 100; b × 200); IHC analysis: The neoplastic cells were positive for S-100 protein (c) and Leu 7 (d) and negative for EMA (e) (× 200)

an embryological remnant terminal nerve and reactive changes as a result of cerebral infarctions or multiple sclerosis [3].

Yasuda [9] reported a case of OECT in 2006 and revealed that OECTs are located in the nerve fiber layer of the olfactory bulb and can promote axonal growth along the primary olfactory pathway of normal adult animals. Similar to Schwann cells, only immunohistochemical staining can identify them. Both Schwann cells and OECTs test positive for S-100 protein and negative for EMA, enabling their differentiation from meningiomas. However, Schwann cells are positive for Leu 7 staining, while OECs are negative [4, 9].

It is extremely difficult to diagnose olfactory schwannoma before surgery due to its uncommonness; most patients are regarded as having olfactory groove meningioma before operation [5]. Histological and IHC analyses, including positive staining for S-100, are vital for pathological diagnosis [4, 9]. Leu7 markers can be used to differentiate OECT from olfactory schwannoma [3–4, 8–9]. Therefore, to improve the diagnostic accuracy, additional IHC staining of Leu7 should be performed.

Our patient who presented vertigo, without headache, anosmia, and seizures was diagnosed based on histological and IHC staining. Pathology of the biopsy specimen showed schwannomas, with the appearance of spindle-shaped tumor cells sparsely and partly arranged in palisades. IHC analysis revealed tumor cells that were positive for S-100 and negative for EMA, similar to OECT as previously published [4]. IHC staining of Leu7(+) suggested an olfactory schwannoma.

The standard treatment of olfactory schwannoma remains unclear due to the disease rarity. As previous literature reported, most cases were treated via a bifrontal craniotomy for surgical excision [1–2, 3, 7–8, 10–11], while some cases underwent an endoscopy-aided or endoscopy resection [5, 12–14]. In our case, we performed a right frontopterional craniotomy approach for surgical resection and obtained a satisfactory surgical result. We reviewed literature where most patients with gross-total resection without postoperative radiotherapy during the subsequent MRI showed no evidence of tumor recurrence [1–2, 11]. However the patient had subtotal resection (STR) whether should have adjuvant radiotherapy still unknown, Kim [8] reported that patients with STR received gamma knife radiosurgery, and within 5 years following radiosurgery, the nasal cavity mass had not grown.

In conclusion, olfactory schwannomas are extremely rare in the anterior skull base; only 67 cases of the olfactory schwannoma have been reported [10]. Although schwannomas are regarded as benign tumors, the prognosis of olfactory schwannoma still needs further studies. Here, we report a patient with olfactory schwannoma to improve its diagnosis and treatment.

Acknowledgments

The authors thank all the patients and their families for participating in this research.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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CASE REPORT

Primary pure squamous cell carcinoma of the duodenum: a case report and review of literature

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Abstract

Primary pure squamous cell carcinomas (SCC) of the duodenum are very uncommon. To the best of our knowledge, only a few cases of SCC of the duodenum exist in the reported literature. Here, we report a case of SCC of the duodenum in northern China. A 45-year-old Chinese Han male patient presented with abdominal pain and weight loss. CT, endoscopic examinations, X-rays, and immunohistochemical markers were used to confirm this rare diagnosis of SCC. We performed a pancreaticoduodenectomy with a curative intention. However, histological examination revealed SCC of the duodenum. Postoperative chemotherapy was started after surgery. To the best of our knowledge, pancreaticoduodenectomy is the preferred form of treatment for carcinoma of the duodenum. This is supplemented with chemotherapy, which can further prolong survival.

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Key words: squamous cell carcinoma; duodenal primary tumor

The duodenum is a unique location for primary squamous cell carcinoma (SCC). Although this tumor was first reported in 1940 in the English language literature [1], there is still little information on this subject with very few articles and case reports. So far, fewer than 10 cases of primary SCC of the duodenum have been reported. Here, we present a case of a 45-year-old male with primary pure SCC of the duodenum.

Case presentation

A 45-year-old Chinese Han man complained of pain in the right upper quadrant and right interscapular region, weight loss of 5 kg, and melena associated with an episode of fever. At the same time, a history of abdominal fullness, discomfort, and jaundice was presented. No evidence of any relevant disease in the family history was found.

Physical examination through abdominal palpation revealed no mass. However, barium swallow exploration confikkrmed the duodenal mass and fistula (Fig. 1). Abdominal ultrasound scan confirmed a good hemodynamic state with an epigastric mass measuring 7.5–8 cm, fixed. Abdominal CT scan highlighted a duodenal tumor with encroachment on the duodenal and infiltration of the surroundings (Fig. 2). Endoscopic examination also showed a circumferential tumor reducing the lumen in the junction of the bulb and descending duodenum.

A cephalic duodenopancreatectomy (Whipple procedure) and resection of the hepatic flexure was performed (Fig. 3). The postoperative recovery of the patient was quick and uneventful. Histological examination showed a primary duodenal SCC moderately differentiated (G2). Keratinization was also observed (Fig. 4). To con¬firm the diagnosis, additional immunohistochemical staining analyses (HCK (+), CK 5/6 (+), and Ki-67 40% (+)) of the duodenal lesion were performed (Fig. 4).

Since patient histology involved SCC, adjuvant treatments of oxaliplatin infusions were elected in combination with oral capecitabine. Patient experienced no complications and is currently following surveillance.

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Discussion

SCC of the duodenum is exceedingly rare and only occasional case reports are seen in the medical literature. Our review of the literature (English) revealed only 9 cases of pure SCC of the duodenum (Table 1) [1–7]. Therefore, little information is available as only a few ca ses present with this diagnosis. Most of the SCC of the duodenum are metastatic tumors from other solid organs such as the cervix, breast, lung, pancreas, and stomach [8].

Since non-specific clinical presentation of SCC can occur, endoscopic examinations are preferred and precise biopsies are recommended in symptomatic patients. CT, ultrasound examinations, X-rays, and immunohistochemical markers were used to confirm this rare diagnosis of primary SCC of the duodenum. This carcinoma is usually localized to the second part of the duodenum [3]. In this case, the imaging investigations did not reveal tumors in other locations and the patient did not present with jaundice. The pathogenesis of SCC of the duodenum is still uncertain. Barnhill and colleagues reported an interesting duodenal tumor with tripartite differentiation into adenocarcinoma, SCC, and neuroendocrine carcinoma. In their case, they speculated that the tumor had arisen from pluripotent duodenal stem cells capable of differentiating into multiple cell types [9]. The tumor in this case may also have arisen from such pluripotent duodenal stem cells. Amjad A reported a patient with Lynch syndrome who was diagnosed with a SCC. This patient's duodenal SCC showed loss of MSH2 and MSH6[5], which suggested a pathogenic role for the MSH2 and MSH6 germline mutation in this tumor.

Diagnosis of SCC of the duodenum depended on CT scans, endoscopic examination, etc. Histopathological examination is the most reliable way to distinguish between the primary and metastatic tumors of the gastrointestinal tract. Pathogenetic differences between metastatic and primary tumors also help clinicians in differential diagnosis[8]. Although no other elements were recognized, malignant squamous cells were positive for keratinization

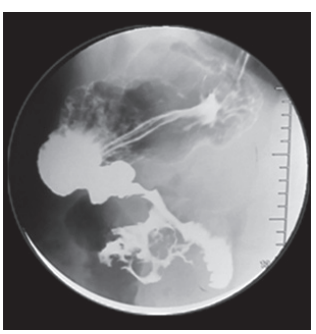

Fig. 1 Barium swallow exploration showing duodenal fistula and widening of the duodenal frame

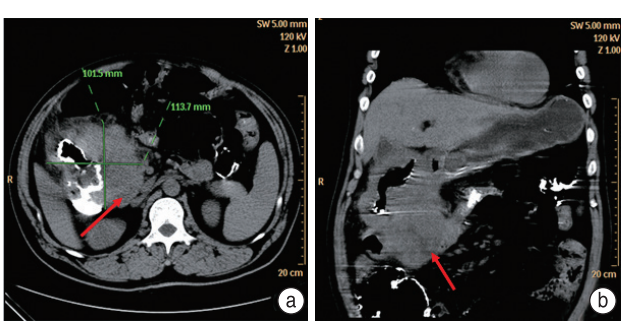

Fig. 2 CT scan. (a) Inhomogeneous duodenal mass (sagittal section); (b) Duodenal tumor (transversal section)

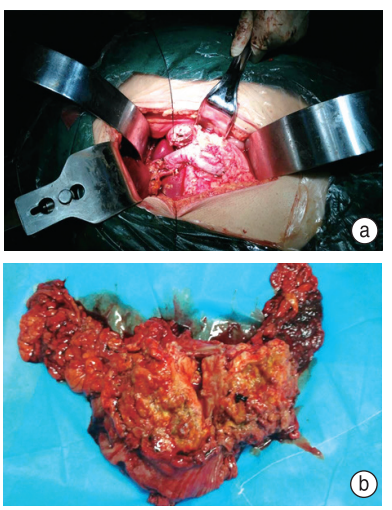

Fig. 3 (a) Intraoperative aspect showing operatory field; (b) Macroscopic view of the mass

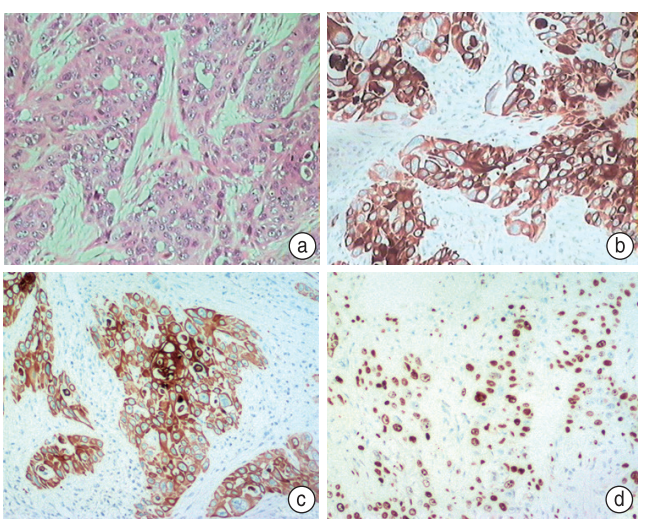

Fig. 4 (a) Pure squamous cell carcinoma of the duodenum with keratinization (HE staining × 200); Immunohistochemistry positive for CK5/6. Photomicrograph of CK5/6 (b), HCK (c), and Ki67(d) staining shows diffused staining of the tumor cells

Table 1 Review of clinical characteristics of patients from published case series of duodenal squamous cell carcinoma

Ref#	Time	Author	Age (years)	Gender	Sites	Treatment	Outcome
1	1986	Friedman E	61	Male	Transverse duodenum	Partial duodenectomy	NR
2	2006	von Delius S	75	Female	Duodenal bulb	NR	NR
3	2009	Terada T	75	Male	Descending part of duodenum	Chemotherapy + radiation	Died 17 months latter
3	2009	Terada T	58	Female	Descending part of duodenum	Chemotherapy +radiation	Died 21 months later
3	2009	Terada T	54	Male	Descending part of duodenum	Surgery	
4	2012	Diffaa A	60	Female	Transverse duodenum	Palliative chemotherapy	Died 1 month later
5	2014	Amjad A	58	Male	Duodenum	Surgery (distal gastrectomy and BillrothII gastrojejunostomy) Chemotherapy (5-fluorouracil+cisplatin)	Surveillance
6	2014	Graur F	47	Female	Descending part of duodenum	Duodenopancreatectomy	NR
7	2015	Battal M	39	Male	Transverse duodenum	Surgery	Surveillance

and intercellular bridges. Immunohistochemistry may also be useful in making the right diagnosis [10]. Similar to this method, CK7, CK20, and TTF-1 levels have also been widely used to distinguish pulmonary carcinomas from gastrointestinal carcinomas [8]. CK5, p63, and p16 were previously selected as immunohistochemical markers to successfully diagnose SCC of the cervix that had metastasized to the duodenum[11]. Distinguishing between the two requires extensive evaluation, including patient clinical history evaluation, histological examination, immunohistochemical analysis, and possibly microarray data analysis.

Conclusion

The optimal treatment and prognosis of SCC of the duodenum is elusive because of the rarity of this disease. Endoscopic and radiological evaluations can prove insufficient to distinguish between benign and malignant tumors. Thus, extensive surgery may be required. In our patient, the tumor was localized to the duodenum with negative margins of resection. Resected lymph nodes were also negative. The metastatic work-up did not show any other primary focus of the disease. Follow-up needs to be more frequent to detect possible early recurrences or distal metastases.

Acknowledgements

We wish to thank our patient and his family for consenting to the publication of this case report.

Ethics approval and consent to participate

The patient described in this case report agreed to the information being used for publication.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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CASE REPORT

Superior mesenteric venous thrombosis after laparoscopic radical resection of rectal cancer: a report of a rare case and literature review*

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Abstract

Mesenteric venous thrombosis (MVT) is rare, but life-threatening. MVT is often characterized by occult and nonspecific signs and symptoms. Diagnosis requires a high index of clinical suspicion, and emergency surgery is necessary to optimize patient survival, especially in people aged more than 70 years. MVT is a rare but fatal complication after laparoscopic radical surgery for rectal cancer. This study reports a case of extensive intestinal ischemic infarction caused by acute MVT after laparoscopic radical surgery for rectal cancer in a 70-year-old male.

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Key words: venous thrombosis, mesentery; radical resection of rectal cancer; intestinal necrosis

Colorectal cancer is the third most commonly diagnosed cancer in men and is second most common in women, representing almost 10% of the annual global cancer incidence [1]. Large comparative studies and multiple prospective randomized controlled trials (RCTs) have reported short-term and long-term equivalence of open surgical and laparoscopic treatment for colon cancer, leading to wide acceptance of laparoscopic colon cancer resection [2]. Laparoscopic resection is safe and reliable for low colorectal cancer, increasing the chance of recovery of anal function [3]. However, serious postoperative complications can occur, such as mesenteric venous thrombosis (MVT). This refers to acute, subacute, or chronic thrombosis of the superior mesenteric vein or branch. It is considered a rare and insidious disease with a high mortality rate, and may present with acute abdominal pain or asymptomatic incidental findings on abdominal imaging. The estimated annual incidence of MVT is 2.7 per 100,000, and is highest in men (12.0 per 100,000) and women (10. 8 per 100,000 people) 70-79 years old [4]. We report a case of extensive intestinal ischemic infarction caused by acute MVT in a 70-year-old man after laparoscopic radical resection for rectal cancer (LAR).

Case report

A 70-year-old man with rectal cancer underwent LAR in a local hospital. The patient had a history of transient cerebral ischemia 7 years prior. Successful LAR was performed 15 days prior to transfer. The patient passed flatus on the third day after surgery and defecated on the fourth day He recovered without incident, and started a Chinese medicine preparation on the fifth postoperative day. He then developed abdominal pain and distension and decreased flatus, and had no bowel movement for 10 days. One day prior to transfer, the abdominal pain

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and distension worsened. He was transferred to the emergency department of the Affiliated Hospital of Qingdao University. Physical examination revealed diffuse abdominal tenderness. His height and weight were 175 cm and 80 kg, respectively, (body mass index: 26.4 kg/m^2). He had not history of diabetes mellitus, hypertension, or a coagulopathy, and was a nonsmoker. The white blood cell count was 23,940/mm^3, and the C-reactive protein level was 7 mg/dL (normal range 0–5). Contrast-enhanced abdominal computed tomography showed a thrombus extending from the superior mesenteric vein to the hepatic portal vein (Fig. 1). MVT with portal vein thrombosis and intestinal obstruction were diagnosed based on the presenting symptoms, signs, and physical and imaging examinations. Preliminary treatment was as follows: (1) Heparin administration by intravenous infusion, with monitoring of blood indicators, and activated partial thromboplastin time maintained at 1.5–2.5 times; (2) Implantation of an indwelling gastric tube for decompression, with drainage of about 200 ml of dark green liquid; (3) Administration of imipenem + vancomycin; (4) Rehydration, with maintenance of water and electrolyte homeostasis.

On the third day after transfer, abdominal distension and pain worsened. Physical examination revealed abdominal distension, with mild rebound tenderness but no rigidity. Murphy's sign was negative. Tympanic sounds were localized to the mid-abdomen and were thought to be caused by intestinal obstruction. The indwelling gastric tube maintained decompression, and had drained 750 mL of dark green stomach content. With abdominal pain caused by intestinal obstruction, the possibility of progression to ischemic intestinal necrosis could not be ruled out. After consultation with the patient and his family, emergency laparotomy was performed. A 70 cm segment of ischemic small bowel that began at 30 cm from the ligament of Treitz was resected (Fig. 2–3). Partial small intestine resection + jejunal feeding tube implantation were performed, and a double cannula was placed above the anastomosis. The postoperative vital signs were stable (Fig. 4). He was discharged on the 7th postoperative day on oral anticoagulation (warfarin 5 mg/day) for 6 months.

Discussion

The estimated annual incidence of MVT is 2.7 per 100,000, and is highest in men (12.0 per 100,000) and women (10. 8 per 100,000 people) 70–79 years old [4]. Low awareness among clinicians may be responsible for the mortality rate of 20% in recent series. Because the onset of disease may be insidious and follows a benign course, computed tomography is the first choice for primary screening of MVT. Timely diagnosis could enable effective anticoagulation therapy [5–6]. Diagnosis of mesenteric ischemia caused by venous disease requires investigation for risk factors, based on clinical assessment, laboratory testing, and imaging. However, there are some nonspecific

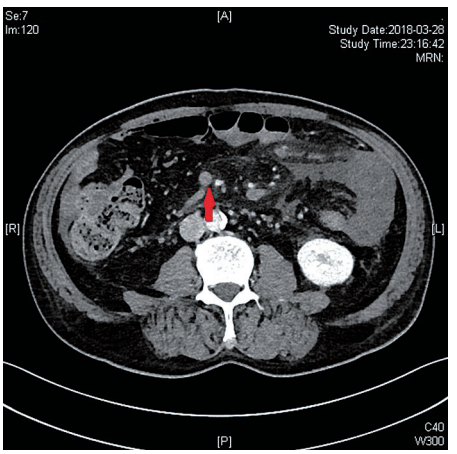

Fig. 1 Abdominal tomography. The red arrow shows the portomesenteric thrombosis

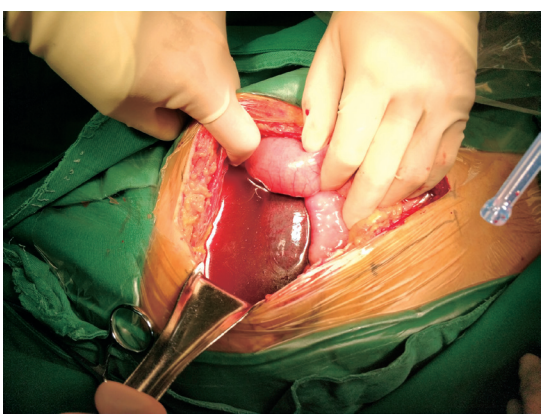

Fig. 2 Intraoperative view: about 1,200 mL of serosanguinous ascites fluid

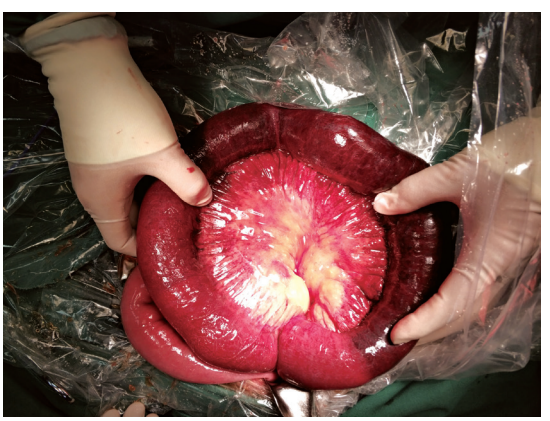

Fig. 3 Image of the ischemic bowel segment

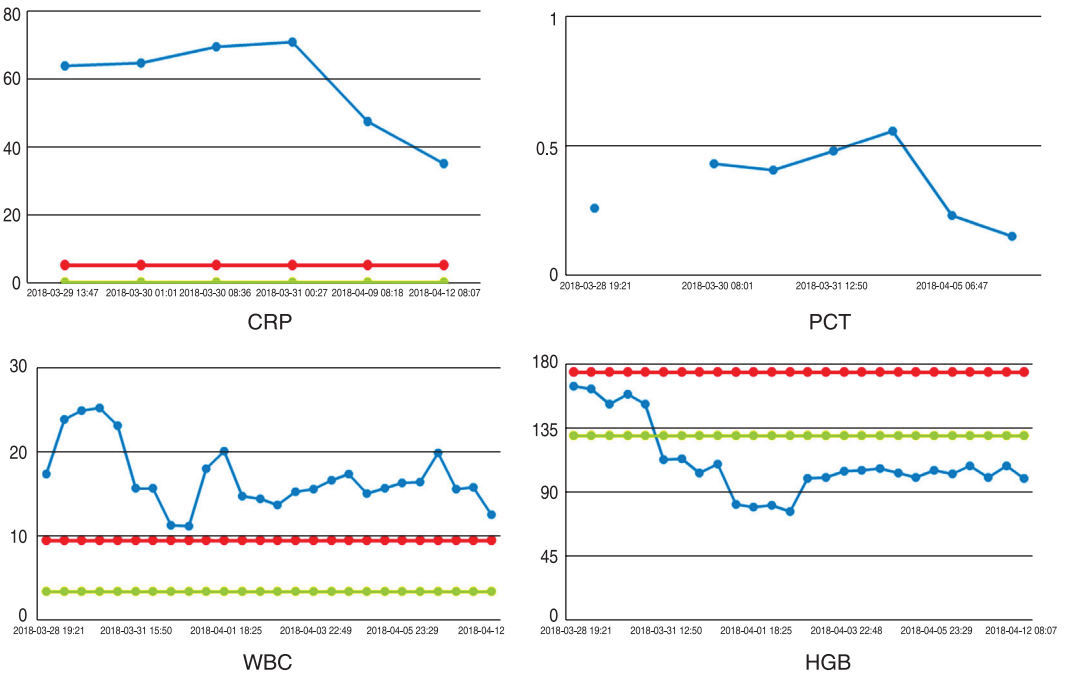

Fig. 4 Postoperative laboratory results

plasma biomarkers of MVT. An elevated D-dimer level may be sensitive but nonspecific [7]. Watershed areas of splanchnic circulation are more vulnerable to ischemia. MVT involves the superior mesenteric vein in 95% of cases and is usually caused by systemic coagulation disorders [8]. MVT may be idiopathic, but is more often secondary to predisposing hypercoagulable states, such as bowel obstruction, inflammation, trauma, major cardiovascular surgery, portal hypertension, and malignancy. MVT secondary to disseminated cancer usually occurs with, but is not limited to, pancreatic adenocarcinoma and hepatocellular carcinoma [9–10]. Thrombi usually originate in the venous arcades and propagate to involve the arcuate channels. Thrombosis of small veins draining close to the bowel is more likely to cause bowel infarction as well. Hemorrhagic infarctions occur when the intramural vessels are occluded. With MVT, bowel ischemia may be acute, subacute, or chronic, and the clinical presentation varies from relatively asymptomatic to acutely ill [11]. The incidence of chronic MVT may be underestimated. MVT is often the result of multiple factors including a hypercoagulable state, endothelial damage, and blood stasis. Hypercoagulable states are divided into hereditary and non-hereditary types. MVT may be due to intestinal inflammation, abdominal infection, or abdominal trauma, and abdominal surgery can lead to endothelial damage and inflammation. Portomesenteric venous thrombosis following laparoscopic surgery usually manifests as nonspecific abdominal pain. Computed tomography can readily provide the diagnosis and demonstrate the extent of the disease. Treatment should be individualized based on the extent of thrombosis and the presence of bowel ischemia but should include anticoagulation therapy. Venous stasis from increased intra-abdominal pressure, intraoperative manipulation of splanchnic vasculature, and systemic thrombophilic states likely converge to produce this potentially lethal condition [12].

This case report suggests that despite advances in minimally invasive techniques, the artificial pneumoperitoneum used in laparoscopic surgery can lead to a sharp increase in abdominal pressure, extensive visceral vasoconstriction, slow flow in mesangial veins, and even stasis, while intraoperative manipulation can lead to visceral vascular endothelial damage and systemic thrombosis. Both can promote the formation of venous thrombosis. Therefore, patients with a previous history of surgery (such as laparoscopic surgery, splenectomy) and risk factors of hepatitis, cirrhosis, venous thrombosis, long-term oral contraceptive use, and malignant tumors should be considered at risk of MVT.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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GUIDELINE OBSERVATION

Updates of the NCCN guidelines for non-small cell lung cancer

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Updates in version 2.2019 of the NCCN guidelines for non-small cell lung cancer from version 1.2019

NSCL-22

ALK rearrangement positive metastatic NSCLC: Lorlatinib added as a treatment option after progression on crizotinib and alectinib, brigatinib, or ceritinib.

NSCL-23

ALK rearrangement positive metastatic NSCLC: Lorlatinib added as a treatment option, after progression on alectinib, brigatinib, or ceritinib.

NSCL-24

ROS1 rearrangement positive metastatic NSCLC: Lorlatinib added as a treatment option, after progression on crizotinib or ceritinib.

Updates in version 1.2019 of the NCCN guideliynes for non-small cell lung cancer from version 6.2018

DIAG-2

Footnote g modified: "Non-solid (ground-glass) nodules may require longer follow-up to exclude indolent adenocarcinoma." (also applies to DIAG-3).

DIAG-3

Solitary pure ground-glass nodules ≥ 6 mm.

Follow-up modified: "CT at 6–12 mo to confirm, no growth or change in solid component, then CT every 2 y until 5 y".

Solitary part-solid nodule(s); sub-categories modified: "< 6 mm", "≥ 6 mm".

Follow-up modified: CT at 3–6 mo to confirm, no growth or change in solid component, then annual CT for 5 y.

DIAG-A 3 of 3

Bullet added: "An EBUS-TBNA negative for malignancy in a clinically (PET and/or CT) positive mediastinum should undergo subsequent mediastinoscopy prior to surgical resection." (also added to footnote h on NSCL-2).

Bullet modified: "TTNA and anterior mediastinotomy (ie, Chamberlain procedure) provide additional access to anterior mediastinal (station 5 and 6) lymph nodes if these are clinically suspicious. If TTNA is not possible due to proximity to aorta, VATS biopsy is also an option".

NSCL-2

Durvalumab changed from a category 2A to a category 1 recommendation. (also applies to NSCL-5, 6, 8, 11, 12, E)

Footnote k added: "If MRI is not possible, CT of head with contrast." (also applies to NSCL-4, 7, 9, 11, 12, 13, 16).

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