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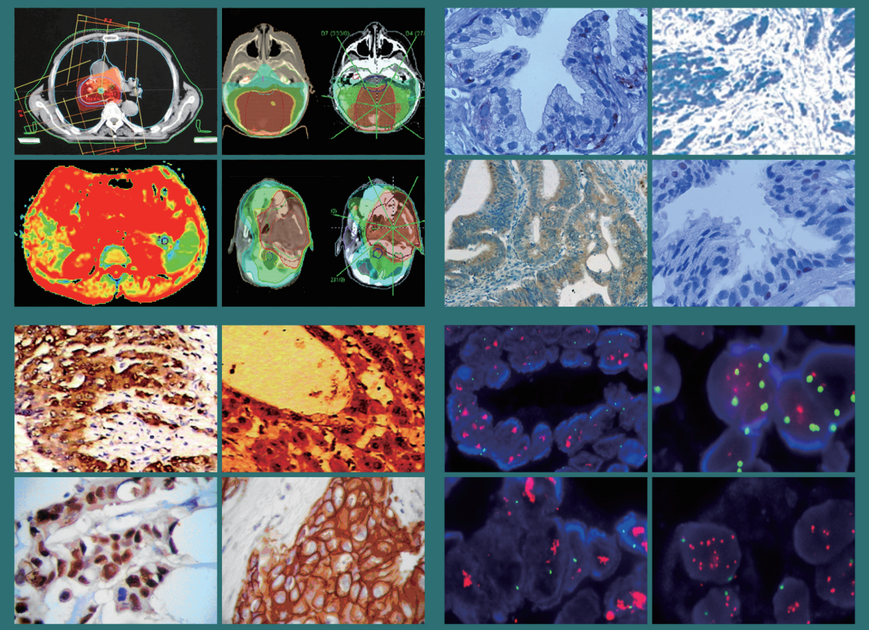

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Aims & Scope

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REVIEW ARTICLE

Advances in the diagnosis and treatment of patients with cancer cachexia

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Abstract

Cachexia is a common complication with an incidence rate of 50%–80% in cancer patients. It is also responsible for 20% of mortality among these patients. Cachexia can significantly reduce the efficacy of antitumor therapies and increase treatment-related toxicity and adverse effects in cancer patients. This increases the symptom burden in patients, affects their quality of life, and ultimately shortens their survival time. The mechanism underlying the development of cachexia is complex and diverse and involves various factors and pathways, each playing an important role. Treatment approaches for cachexia are multimodal, including nutrition support therapy, appetite stimulants, and therapeutic drugs that specifically target the mechanism behind the disease. In recent years, we have gradually gained a better understanding of cachexia, and significant progress has been made in delineating molecular mechanisms, staging and diagnosis, and therapeutic drug treatment of cancer cachexia. This article reviews the research progress of cancer cachexia based on these contexts.

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Key words: cachexia; malignant tumor; molecular mechanism; staging and diagnosis; treatment

Definition and diagnosis of cachexia

Cachexia is a complex metabolic syndrome that threatens patients' lives. It is characterized by weight loss and muscle wasting with or without fat loss. The pathophysiological characteristics of cachexia include weight loss, anorexia, inflammation, insulin resistance, muscle protein breakdown, and fat decomposition [1–2]. Cachexia is most commonly seen in various chronic consumptive diseases, such as chronic obstructive pulmonary disease, rheumatoid arthritis, chronic kidney disease, chronic heart failure, AIDS, and malignant tumors [3–8]. Cancer cachexia, also known as cancer anorexia cachexia syndrome (CACS), has an incidence rate of approximately 50%–80% in patients with various types of cancer. Of all cancer types, the incidence rate of cachexia is the highest in pancreatic cancer and upper gastrointestinal cancer patients (> 80%), followed by lung and colon cancers, wherein approximately 50%–60% of patients develop cachexia [9–10]. Among the different causes of death, cachexia is responsible for 20%–40% of deaths in cancer patients [11–12]. Many previous studies have shown that cachexia not only reduces the efficacy of antitumor therapies and increases treatment-related toxicity and adverse effects but also increases the symptom burden in patients, reduces their quality of life, and ultimately shortens their survival time [13–17].

Despite the complex and diverse mechanisms involved in the development of cachexia, a precise and standardized definition for cachexia is still lacking. Moreover, the identification, diagnosis, and treatment of cachexia are often neglected in the clinical setting [18–19]. In a consensus meeting held in Washington D.C. in 2006, experts unified the definition of cachexia: a complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss of fat mass [20]. In 2011, the international expert consensus set the diagnostic criteria for cachexia: a patient is diagnosed with cachexia if in the past 6 months, weight loss was greater than 5% or 2% in individuals with body mass index (BMI) of less than 20 kg/m^2 or those with sarcopenia [21]. This definition has since become widely accepted and adopted by a

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number of clinical studies on cachexia [22–24].

Molecular mechanism of cachexia

Muscle wasting is one of the important features of cancer cachexia, and its pathophysiology is characterized by an imbalance in the synthesis and degradation of muscle proteins. Currently known cytokines and molecular mechanisms involved in cachexia-induced muscle wasting are summarized below.

Systemic inflammation

Systemic inflammation is the main mechanism leading to muscle wasting and fatigue in patients with cachexia [25]. Early studies on the mechanism of cachexia have principally focused on inflammation. The pro-inflammatory factors produced by the body or the tumor, including TNF-α, IL-1, and IL-6, are closely related to muscle wasting in cancer cachexia [26–28]. Many studies have shown significantly increased inflammatory markers in the blood of cachectic animal models and patients [29–32]. Earlier studies have considered TNF-α as a major factor that induces cachexia. It has been shown to cause muscle protein breakdown and muscle atrophy in animal experiments [33–34]. TNF-α and IL-1 induce cachexia through the activation of IKK complexes, which leads to the phosphorylation of the IκBa protein and the release of NF-κB. This activates the muscle-degrading factors MuRF1 and Atrogin-1, resulting in protein loss and muscle atrophy [35–36]. IL-6 induces cachexia through binding to IL-6 receptors, which activate the downstream JAK-STAT pathway. Animal experiments have shown that STAT3 can cause muscle fiber atrophy and that the IL-6/JAK-STAT3 pathway is closely related to skeletal muscle atrophy [37].

Ubiquitin proteasome pathway (UPP)

The UPP is an important pathway for muscle degradation in cachexia [38–39]. The majority of muscle proteins, particularly muscle fibers, are degraded by the UPP. The degradation is generally divided into two steps: the substrate protein is first covalently bound to different types of ubiquitin molecules and is then degraded by the 26S protease. The process of protein ubiquitination is usually regulated by three enzymes: ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3) [40]. Atrogin-1 and MuRF-1 are two important E3 ubiquitin ligases. A marked increase in the expression of Atrogin-1 and MuRF-1 has been observed in cachexia, and their expression is correlated with muscle atrophy [41–42]. Many animal experiments have shown that cancer cachexia can significantly increase the activity of the ubiquitin proteasome system (UPS), resulting in increased expression of Atrogin-1 and MuRF-1 [43–45].

PI3-K/Akt/mTOR pathway

The IGF-1 signaling pathway is an important pathway involved in muscle anabolism. Studies have shown that the IGF1/Akt pathway can inhibit protein degradation and promote muscle growth [46–47]. In addition, binding of IGF1 to the receptor can activate the PI3K/Akt signaling pathway. This activates mTOR and phosphorylates its effector targets S6K1 and 4E-BP, which in turn promote muscle formation [48–49]. Akt can also translocate FoxO proteins (FoxO1, FoxO3, and FoxO4) from the nucleus to the cytoplasm, leading to their phosphorylation and inactivation. Activated FoxO proteins can act as transcription factors and regulate autophagy, which promotes the ubiquitin-mediated degradation of muscle cells [50–52]. IGF-1 expression is significantly reduced in animal models of cancer cachexia, and supplementation with low-dose IGF-1 can reduce muscle atrophy and weight loss. However, anti-IGF-1 treatment has not been shown to exacerbate muscle atrophy in cancer patients [45, 53–54].

TGF-β/SMAD pathway

The TGF-β superfamily is another factor that has been recently found to be associated with muscle atrophy in cachexia. The most representative family members are activin A and myostatin [55]. Activin A is implicated in many physiological functions, including erythrocyte formation, cell growth, differentiation, and immune response [56]. Myostatin, also known as GDF8, is an important negative regulator of muscle growth and is secreted by muscle cells. Its deletion and mutation are associated with the pathological condition of muscle hypertrophy [57–58]. Both activin A and myostatin activate type I receptors by binding to the ActRIIB receptor on the surface of muscle cell membranes (ALK4 or ALK7 is an activin A type I receptor, while ALK5 or ALK7 is a myostatin type I receptor). The activated type I receptors, in turn, phosphorylate the SMAD complexes (SMAD2, SMAD3, and SMAD4) and cause muscle atrophy by regulating transcriptional responses [59–60]. Myostatin and activin A can also activate FoxO3 by suppressing Akt activity, which in turn upregulates MuRF-1, Atrogin-1, and autophagy-related genes, leading to the breakdown of muscle protein [61]. It has been observed in animal experiments that elevated activin A expression is associated with muscle wasting in cachexia. In addition, the inhibition of activin A can reduce muscle wasting and improve muscle function. The levels of activin A in the blood of patients with cancer cachexia have also been shown to be significantly elevated [62–64]. The myostatin/activin A/SMAD pathway may be present early in cachexia. A study on patients with early stage gastric cancer detected increased expression of myostatin in patients' muscles prior to their significant weight loss.

This suggested that myostatin might be a marker for early-stage cachexia [65]. However, studies on myostatin and muscle atrophy have reported inconsistent results. Some studies have shown that the increased expression of myostatin in muscles is associated with cancer cachexia-induced muscle atrophy, and the inactivation of the myostatin gene can inhibit muscle atrophy and tumor growth. By contrast, some studies have shown that the expression of myostatin in the serum is not associated with muscle loss [66–70].

GDF-15, also known as macrophage migration inhibitory factor 1, is another member of the TGF-β superfamily. Its hematologic level is significantly elevated in inflammation, cancers, and cardiovascular diseases [71]. Many studies have shown the increased expression of plasma GDF-15 in cancer patients, which is associated with their poor prognosis [72–74]. At the same time, GDF-15 levels are correlated with appetite. An increase in GDF-15 levels in the blood leads to a decreased appetite, which in turn causes weight loss [75]. The overexpression of GDF-15 in the muscles of experimental animals causes muscle atrophy; therefore, GDF-15 may directly promote skeletal muscle atrophy. In cancer patients, the high expression of GDF-15 is associated with weight loss and muscle loss; however, no correlation between GDF-15 and the appetite of patients has been observed [76].

Autophagy-lysosome pathway

Autophagy is a normal, ubiquitous catabolic process that degrades cytoplasmic components through lysosomes, and this process also occurs in skeletal muscles. When occurring properly, autophagy can help regulate the function of skeletal muscles and control skeletal movement and muscle metabolism. However, excessive activation of or deficiency in the autophagy function can result in muscle wasting and reduced muscle function [77–80]. Some studies using animal models of cachexia have shown that autophagy is significantly activated in the muscles of mice with cachexia [81]. The activation of the autophagy pathway has also been observed in the muscle or blood of patients with cancer cachexia, and autophagy is found to be significantly associated with muscle wasting and weight loss [82–84]. It is speculated that aerobic exercise and megestrol acetate may relieve the symptoms of cachexia-induced muscle atrophy by suppressing the excessive activation of autophagy and restoring the balance of muscle metabolism [85–86].

Staging and diagnosis of cachexia

The international expert consensus of cachexia [21] has divided the development and progression of cachexia into three consecutive phases: precachexia, cachexia, and refractory cachexia. Patients with precachexia usually present with clinical or metabolic symptoms, including anorexia and impaired glucose tolerance, accompanied by weight loss of ≤ 5%. A patient enters the cachexia phase if weight loss exceeds 5% or 2% for patients with BMI of less than 20 kg/m^2 or those with sarcopenia. Weight loss may occur under the influence of factors such as tumor type and stage, systemic inflammation, reduced food intake, and ineffective antitumor therapy. In refractory cachexia, the patient is usually at the end stage of cancer, with a performance status score of 3–4. The tumor progresses rapidly and is unresponsive to antitumor therapy, and the patient has an expected survival time of less than 3 months. Although the international expert consensus has set the definitions and descriptions for cachexia stages, to date, widely accepted criteria for staging cachexia are still lacking. In addition, staging of cachexia is crucial for treatment selection and prognosis of patients.

In 2009, Bozzetti F *et al* [87] classified cachexia into precachexia and cachexia based on the presence of 10% weight loss. They further classified the disease into asymptomatic precachexia (stage I), symptomatic precachexia (stage II), asymptomatic cachexia (stage III), and symptomatic cachexia (stage IV) based on the presence of anorexia, fatigue, or early satiation. This staging methodology preceded the development of the diagnostic criteria for cachexia by the international expert consensus and hence adopted a 10% weight loss as a diagnostic criterion. Furthermore, it lacks a diagnostic criterion for refractory cachexia. In 2011, Argiles JM *et al* developed a new tool for staging cachexia (CASCO) [88]. It included five major diagnostic indicators: body weight and muscle changes, inflammation/metabolic disturbances/immunosuppression and related parameters, physical performance, nutritional status, and quality of life. The total score of the scale is 100 points. It divides cachexia into mild (0–25 points), moderate (26–50 points), severe (51–75 points), and terminal phase (76–100 points). However, the scoring table contains a large number of questionnaires and metabolic and immunologic parameters. Its complexity and high cost limit its widespread use in clinical settings. Vigano A *et al* subsequently introduced a novel definition for staging cancer cachexia (CCS) [89] that comprehensively determined cachexia stages based on parameters such as inflammatory indicators, anorexia, weight loss, physical performance, and grip strength. However, their staging criteria failed to properly distinguish patients with precachexia and cachexia. In 2014, Blum D *et al* conducted a validation study on the international expert consensus on cachexia [90], in which patients were classified into different cachexia stages according to the degree of weight loss: patients with weight change (± 1 kg) or weight gain were classified as no cachexia; patients with weight loss > 1 kg but < 5% were classified as precachexia; patients

with weight loss > 5% or patients with a BMI < 20 kg/m^2 and weight loss > 2% were classified as cachexia; and patients with a BMI < 23 kg/m^2 with weight loss > 15% or those with a BMI < 27 kg/m^2 and weight loss > 20% were classified as refractory cachexia. However, weight loss alone cannot properly reflect the status of cachexia in patients. In addition, it cannot distinguish between patients without cachexia and those with precachexia. In 2016, Vigano AA *et al* optimized previous CCS criteria and developed a simple, clinically applicable system for staging of cachexia. Five indicators were used for staging and diagnosing cachexia, including abnormal biochemical parameters, reduced food intake, moderate weight loss, severe weight loss, and reduced performance status [91]. Although this staging system is simpler to use than the previous CCS criteria, as it eliminates the need to fill out questionnaires and measure grip strengths, it still cannot effectively distinguish between patients with precachexia and those with cachexia. Similarly, in 2017, Argiles JM *et al* validated and simplified the previously developed CASCO cachexia staging criteria into a new set of cachexia staging criteria (miniCASCO) [92]. Although miniCASCO is more convenient than CASCO, it still requires a large number of questionnaires and parameter testing such as that for IL-6 and ROS. Therefore, it is not suitable for rapid clinical diagnosis. Furthermore, its effectiveness has not been verified in clinical settings. Our research group recently developed a cachexia staging score (CSS) [93], which included five components for evaluation: weight loss, a questionnaire for sarcopenia SARC-F, performance status, appetite loss, and abnormal hematologic parameters. The total score was 12 points, of which 0–2 points were classified as non-cachexia, 3–4 points as precachexia, 5–8 points as cachexia, and 9–12 points as refractory cachexia. The simple design and low cost of this scoring tool facilitate its rapid clinical application. Its effectiveness has also been verified using various clinical parameters, including patients' body weight loss, BMI, muscle mass and function, proportion of sarcopenia cases, symptom burden, quality of life, and survival time. These results indicate that the scoring tool performs well in distinguishing patients with different stages of cachexia.

Advances in the treatment of cachexia

With the extensive research on the molecular mechanism of cachexia in recent years, significant progress has been made in the treatment of cachexia. Many novel drugs have shown therapeutic prospects for cachexia. As the mechanism underlying the development of cachexia is complex and diverse, a single treatment approach can hardly achieve satisfactory results. Therefore, cancer cachexia is best treated with comprehensive multimodal therapies. This section provides a summary of the main treatment approaches for cancer cachexia.

Nutrition support therapy

Weight loss and malnutrition are the most common signs of cancer cachexia that can adversely affect patients' clinical outcomes. Therefore, it is necessary to perform appropriate nutritional screening for cancer patients. Additionally, the advantages and disadvantages of nutritional intervention need to be weighed and properly balanced [94]. In clinical practice, nutrition support therapy is usually the most considered treatment for patients with cachexia. However, with deeper understanding of cachexia, we now realize that nutrition support therapy may not be applicable to all patients with cachexia. In addition, nutrition support therapy alone cannot completely alleviate patients' symptoms of cachexia. The international expert consensus on cachexia has pointed out that nutrition support therapy may not be beneficial to patients with refractory cachexia [21]. Therefore, guidelines in the United States do not recommend the routine use of nutrition support therapy in cancer patients receiving chemotherapy or minor surgery. According to the guidelines, nutrition support therapy should only be considered in patients who are unable to absorb adequate nutrients due to functional impairment [95]. Among the various nutritional supplements, n-3 polyunsaturated fatty acids have been shown by many studies to be beneficial to cancer patients, and they can increase their weight and improve their quality of life [96–98]. In addition, L-carnitine has been shown to alleviate fatigue while improving the nutritional status of cancer patients. However, other studies have obtained contrasting results [99–102]. Therefore, the use of nutritional supplements in patients with cachexia remains inconclusive.

Appetite stimulants

Appetite stimulants commonly used in patients with cancer cachexia include hormones and progesterone [2]. A systematic review has revealed that while hormones and progesterone drugs are recommended for the treatment of anorexia in cancer patients, there are uncertainties regarding their appropriate dose, timing, and treatment duration [103]. Hormonal drugs are often used as appetite stimulants to improve appetite, increase caloric intake, control pain, alleviate fatigue, and reduce nausea and vomiting of cancer patients [104–105]. Various hormonal drugs exert similar appetite-stimulating effects. The commonly used hormonal drugs include prednisone and dexamethasone. Studies have shown that 5 mg of prednisone administered orally three times per day and 3–6 mg of dexamethasone administered orally per day

can significantly increase patients' appetite compared with placebo [106]. However, hormonal drugs can only increase the appetite of patients for a short period of time; they cannot truly increase the weight of patients [107–108]. In addition, as hormone therapy is associated with many adverse effects that can negatively affect the patient's quality of life, the dosing and timing of hormonal drugs require careful monitoring [109].

The most common progesterone used clinically as appetite stimulants include megestrol acetate and medroxyprogesterone acetate. The appetite-stimulating effects of megestrol acetate are similar to those of dexamethasone. Several clinical studies have shown that megestrol acetate can significantly improve the appetite of cancer patients while having milder adverse effects compared with dexamethasone [110–112]. Medroxyprogesterone can also increase the appetite of cancer patients and increase their body weights. However, the increase is limited to adipose tissue, not muscle tissue [113–116].

Thalidomide

Thalidomide possesses immunomodulatory and anti-inflammatory effects. Hence, it can reduce the level of inflammatory factors (TNF-α and IL-6) in the blood, thereby inhibiting the NF-κB pathway and reducing cachexia [117–118]. Studies have shown that thalidomide has a positive therapeutic effect on cancer cachexia. However, some studies have reported that patients treated with thalidomide do not show a significant decrease in symptom severity and inflammatory parameters compared with the placebo groups. Therefore, the effectiveness of thalidomide for treating cancer cachexia will need to be confirmed by data collected from large-cohort randomized controlled trials [119–123].

Selective COX-2 inhibitors

Selective COX-2 inhibitors are anti-inflammatory drugs that can be used for the treatment of cachexia [124]. Phase II clinical studies have shown that when used in combination with other drugs, celecoxib can significantly increase the lean body mass, grip strength, quality of life, and performance status of cancer patients. It can also reduce the level of TNF-α in the blood and does not cause grade 3–4 adverse reactions [125–126]. However, the latest research shows that when used in combination with megestrol acetate, celecoxib cannot further enhance its efficacy in the treatment of cachexia [127].

TNF-α inhibitors

As TNF-α plays an important role in the development and progression of cachexia, therapeutic drugs that inhibit TNF-α may be beneficial for the treatment of cachexia [128–129]. It has been shown in animal experiments that TNF-α inhibitors significantly increase the appetite and body weight of tumor-bearing mice. Infliximab is a human and mouse chimeric monoclonal antibody that specifically blocks TNF-α. However, multiple phase II clinical studies have shown that infliximab fails to alleviate muscle atrophy or improve the quality of life of patients compared with the controls [130–133]. The above findings suggest that the mechanism underlying the development of cachexia can be diverse. Therefore, a single treatment modality can hardly produce satisfactory results, and the treatment of cachexia requires comprehensive multimodal therapies. Moreover, a phase II/III randomized controlled study on infliximab in lung cancer patients was prematurely terminated due to a significant reduction in quality of life in the treatment group.

In addition to TNF-α receptors, fibroblast growth factor-inducible 14 (Fn14), a receptor for TWEAK, is also a member of the TNF receptor superfamily. Fn14 has been shown to be related to the mechanism of cancer cachexia development [134–136]. Monoclonal antibodies against Fn14 have been shown to alleviate symptoms of cachexia and prolong survival in mice, whereas anti-TWEAK antibodies have no therapeutic effects on the Fn14-induced cachexia, suggesting that there may be another unknown ligand for Fn14 [137].

IL-6 receptor inhibitor

ALD518 is a humanized monoclonal antibody with high affinity toward IL-6. It is used in the treatment of anemia, cachexia, and asthenia [26, 138]. In a phase I clinical study, ALD518 has been shown to improve grip strength and fatigue in patients with advanced tumors [139]. A subsequent phase II randomized controlled trial in patients with advanced non-small-cell lung cancer (NSCLC) showed that compared with the control group, ALD518 significantly reduced body weight loss, alleviated lung symptoms, and improved fatigue and anemia in the treatment group [140–142]. These results indicate that ALD518 is safe and well tolerated. It may serve as a potential therapeutic drug to improve anemia, fatigue, and cancer-associated cachexia. However, its efficacy needs to be further confirmed in large cohorts and phase III randomized controlled clinical trials.

Ghrelin receptor agonist

Ghrelin is a newly discovered growth hormone-releasing peptide that is primarily synthesized in the stomach. It can regulate the release of growth hormone, stimulate appetite, inhibit the production of pro-inflammatory factors, and regulate energy fluxes in an organism [143–144]. Studies in animal models of cachexia and human patients with cancer cachexia have shown that ghrelin can significantly increase food intake and body weight in mice or patients with cancer cachexia [145–146]. The recently developed anamorelin is an oral ghrelin receptor agonist. It has been shown in preclinical studies that the administration of 10 or 30 mg/kg of anamorelin in mice can significantly stimulate appetite and increase food intake and body weight [147]. Two subsequent phase II clinical studies showed that continuous administration of anamorelin for 12 weeks significantly increased the lean body mass of patients with cachexia [148]. The results of two phase III randomized controlled clinical trials in patients with NSCLC (ROMANA1 and ROMANA2) showed that anamorelin significantly increased the lean body mass of patients with cancer cachexia, but not their grip strength and muscle function [149]. In a related phase III safety extension study, the use of anamorelin was extended to 24 weeks. The results showed that anamorelin was well tolerated. Additionally, anamorelin significantly increased the patients' body weights and reduced their symptom burden [150]. A recently completed randomized controlled clinical study of anamorelin in Japan also showed that it could increase the lean body mass in patients and alleviate symptoms such as anorexia; however, muscle function was not enhanced [151]. Many meta-analyses and systematic reviews also showed that anamorelin significantly improved the appetite and lean body mass of patients with cancer cachexia, but did not affect their grip strength and overall survival [152–153]. Despite these findings, anamorelin is still currently considered a new option for the treatment of cancer cachexia. Phase III clinical studies of anamorelin in Chinese patients with cancer cachexia are currently ongoing.

ActRIIB antagonists

Many studies have shown that levels of activin A and myostatin are significantly elevated in patients with cancer cachexia [55, 154]. The inhibition of the myostatin/activin A signaling pathway in mouse models of cancer cachexia can increase muscle volume and improve physical performance and muscle function [63, 155–156]. ActRIIB antagonists are inhibitors of the SMAD2/3 pathway, which is mediated by both myostatin and activin A. They can significantly reduce muscle atrophy and prolong survival in animal experiments, but have no effect on the levels of inflammatory factors in the blood [29]. Another myostatin-specific antibody, PF-134, has also been confirmed to reduce tumor-induced muscle atrophy and impaired muscle function in animal experiments. However, a clinical study on PF-134 was terminated due to oral bleeding and epistaxis that occurred during the trial [157]. LY2495655 is another myostatin-specific antibody that has been shown in clinical studies to alleviate muscle atrophy and improve grip strength and muscle function in patients with cancer cachexia. Phase II/III clinical studies on LY2495655 are ongoing [158].

Summary

With our increasing understanding of cancer cachexia in recent years, significant progress has been made in the diagnosis and treatment of cachexia. The international expert consensus has set clear definitions for cancer cachexia that are gradually becoming the accepted diagnostic standards. The staging criteria for cachexia are also continually being refined. Additionally, with the extensive research on the molecular mechanism of cachexia, there have been more promising targeted therapeutic drugs for cachexia. However, the mechanism underlying the development of cachexia is complex and diverse, and a single treatment modality will hardly produce satisfactory results. Many challenges remain in the diagnosis and treatment of cancer cachexia: How can we improve the screening of patients with cancer cachexia in clinics? What are the markers of the development and progression of cachexia? How can we optimize the staging and diagnosis of patients with cachexia? What are the appropriate multimodal treatment plans for cancer patients with different stages of cachexia? Future research should focus on finding solutions to these issues.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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REVIEW ARTICLE

Updates in the management of brain (leptomeningeal) metastasis of lung cancer

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Abstract

Brain (leptomeningeal) metastasis is one of the most common and severe complications of lung cancer. This article interprets expert consensus on the treatment advice for brain (leptomeningeal) metastasis of lung cancer, expounding on its epidemiology, diagnostic standards, efficacy assessment, treatment advice, and other aspects.

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In 2012, there were approximately 1.24 million new cases of lung cancer and 1.1 million related deaths worldwide; in 2016, a total of 220 000 new patients were diagnosed with lung cancer in the U.S. alone, and over 158 000 of them died from the disease. One of the most common and severe complications of lung cancer is brain metastasis (BM). Although there has not been any census of the actual global or national incidence rate of BM, a conservative estimate reveals that 10%–30% of lung cancer patients will experience BM. In the past, the survival rate after BM used to be low, and treatments were often futile. Nevertheless, with the emergence of molecular targeted therapy and immunotherapy, the survival rate of lung cancer has been rising continuously. Consequently, patients also suffer from a greater risk of developing sequelae like BM at the later stages of lung cancer [1]. In the U.S., BM is the most prevalent tumor in the central nervous system (CNS). It may emerge as an initial symptom of cancer before cancer diagnosis or appear within a few years or decades after the confirmatory diagnosis of primary cancer. The incidence rate of BM differs significantly depending on the location of the primary cancer; the main primary cancers related to BM are lung cancer, breast cancer, and melanoma. BM is difficult to treat, and to most individuals, the diagnosis of BM is usually a sign of poor prognosis [2]. Among all patients of solid tumors, the incidence rate of leptomeningeal metastasis (LM) ranges from 1% to 9.1%; over the last decade, lung cancer and breast cancer were the most common primary solid tumors associated with LM [3]. The incidence rate of LM is 3.8% in patients with non-small cell lung cancer (NSCLC), most of whom are females and non-smokers and have adenocarcinoma; one third of the patients already have BM at the time of diagnosis of LM [4].

Diagnosis and classification

LM refers to the multifocal seeding of cancer cells in the leptomeninges [5]. Malignant cells can reach the leptomeninges in several ways: hematogenous spread through arterial or venous circulation, lymphatic spread around blood vessels, dissemination along or around nerves, direct spread of metastatic lesions from the bones or the part of the brain near the arachnoid or interventricular space, as well as from choroid plexus and subependymal metastases. LM is divided into 2 types: diffuse and nodular. The former involves free-floating and non-adherent cancer cells, whereas the latter is characterized by contrast-enhancing leptomeningeal tumor nodules [5].

The diagnosis of LM entails three key elements that are universally recognized: neurological symptom assessment, neuroimaging evaluation, and cerebrospinal

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fluid (CSF) cytology or flow cytometry (FC). The Response Assessment in Neuro-Oncology (RANO) LM working group recommended that all patients enrolled in LM clinical trials should undergo a complete standardized neurological examination, CSF analysis (including cytology for all cancers and FC for hematological cancers), enhanced magnetic resonance imaging (MRI) of the brain and spine, and radioisotope CSF flow studies (only in patients treated with intra-CSF therapy). Most randomized controlled trials related to LM have already adopted a combination of neurological examination and CSF cytology to assess therapeutic efficacy.

Neurological symptom assessment

The initial clinical manifestations may not be typical, and may include cauda equina syndrome, cranial nerve defects, headache, back pain, visual impairment, diplopia, hearing loss, and symptoms of neurocognitive disorders . Symptoms related to increased intracranial pressure may arise at a later stage [6].

Neuroimaging evaluation

Brain and spine MRI is the gold standard in LM imaging evaluation. Brain involvement is observed in 40%–75% of LM cases, whereas spine involvement is seen in 15%–25% of cases. The sensitivity and specificity of MRI for detecting LMs of solid tumors are expected to be 70%–87% and 75%–94%, respectively [7]. Gadolinium-enhanced MRI can increase sensitivity, especially in LMs that are mainly or solely manifested in the cranial nerve.

Any stimulus to the leptomeninges, such as surgery or puncture, can induce local MRI enhancement . Therefore, MRI examinations should be conducted before such operations. It is worth noting that normal MRI results cannot exclude the probability of LM because such results are found in up to 20% of LM cases.

CSF cytology/FC examination

CSF cytological analysis remains the gold standard for LM diagnosis. First-time CSF examination yields a sensitivity of 45%–50%. Usually, two consecutive CSF samples are required for an adequate cytological evaluation [8]. Yet, up to 30% of LM cases produce negative CSF cytology results; their diagnosis is assisted by MRI [9].

There are several ways to increase the sensitivity of cytological analysis, including using tumor marker-immunostaining fluorescence *in situ* hybridization (TM-iFISH), CellSearch, and FC [10]. Direct DNA sequencing of the CSF of NSCLC patients with LM can identify sensitizing and resistant epidermal growth factor receptor (EGFR) mutations and detect the same EGFR mutation subtype as that in the primary tumor despite the absence of malignant cells in the CSF [11].

The diagnosis-specific graded prognostic assessment (DS-GPA) was initially based on four factors found in 1833 cases of NSCLC and BMs from 1985–2005: patient's age, Karnofsky performance score (KPS), presence of extracranial metastases, and number of BMs; the median survival of patients who were surveyed for the development of the DS-GPA from the beginning of BM treatment was 7 months. To design a newer version of the DS-GPA, the Lung-molGPA , data from 2186 patients with NSCLC and newly-diagnosed BM (1521 cases of adenocarcinoma and 665 cases of non-adenocarcinoma) from 2006–2014 were analyzed by researchers; significant prognostic factors included the original four factors used in the DS-GPA index, and the addition of two new factors: EGFR and ALK alterations in adenocarcinoma patients (mutation status was not routinely tested for in non-adenocarcinoma patients). The overall median survival for the cohort in that study was 12 months, and patients with NSCLC-adenocarcinoma and Lung-molGPA scores of 3.5–4.0 had a median survival of nearly 4 years. Patient's age, KPS, presence of extracranial metastases, and number of BMs were once again confirmed as prognostic factors. Positive EGFR and ALK results were also independent prognostic factors and were added to the Lung-molGPA. The more significant factors were scored up to 1.0; the higher the score, the better the prognosis. These factors included a KPS of 90–100 [hazard ratio (HR), 0.6 vs KPS ≤ 70], absence of extracranial metastases (HR, 0.5), EGFR or ALK positive (HR, 0.5 vs negative or unknown EGFR and ALK results). The remaining two factors – patient's age and number of BMs – had a less significant impact (HR, 0.7 and 0.8, respectively), and were scored up to 0.5. Therefore, 4.0 remained as the highest possible score. Table 1 describes the new Lung-molGPA parameters in detail [1].

Efficacy assessment

The metastasis of solid tumors to the CNS, be it BM or LM, differs according to histology and molecular subtypes. Under the action of the blood-brain barrier, anti-cancer therapy with systemic activity at the standard dose may fail to reach the same drug concentration in the CNS. Such differences may exert insignificant effects on certain types of drugs; for instance, although immunomodulatory

Table 1 Summary of the new Lung-molGPA parameters [1]

Prognostic factor	GPA (graded prognostic assessment)		
	0	0.5	1
EGFR/ALK	–	NA	+
Age	≥ 70	< 70	NA
KPS	< 70	70–80	90–100
Extracranial metastases	Present	NA	Absent
Number of brain metastases	> 4	1–4	NA

antibodies cannot pass through the blood-brain barrier, expanding and activated peripheral lymphocytes can enter the CNS. However, this issue may lower the activity of some other drugs in the CNS. During the clinical development of a new drug, if the drug lacks CNS activity and is inappropriately included in the clinical trial design or used to assess CNS metastatic diseases, the common efficacy endpoints may be substantially diminished due to early CNS progression. Conversely, if the drug indeed has CNS activity and is inappropriately excluded from the clinical trial design or used to assess CNS diseases, the collection of data about the benefits for the CNS may be hindered.

It is unreasonable to completely exclude BM patients from the clinical trials for diseases such as NSCLC, breast cancer (HER2 positive or triple negative), and melanoma because that can mean excluding half to two thirds of all patients with stage IV cancers. According to a recent systematic study of 413 trials on systemic medications against advanced NSCLC, 14%–19% of the clinical trials excluded all patients with a history of LM or BM, and 41% of them allowed the enrollment of BM patients who had been treated and were in stable condition. Since many BM patients are often excluded from clinical trials, the existing trials are unable to demonstrate efficacy for the treatment of BM [12].

A measurable disease is defined by the presence of contrast-enhanced lesions that can be accurately measured in at least one dimension. The longest diameter in the plane of measurement is to be recorded, and the corresponding perpendicular diameter should also be at least 5 mm long. If the MRI is performed with thicker slices, the size of the measurable lesion at baseline should be at least two times the slice thickness. When determining the minimum size of the measurable lesion at baseline, the presence of inter-slice gaps should also be taken into consideration.

Non-measurable lesions include: those with a longest diameter of less than 10 mm, those with boundaries that are not repeatedly measurable, dural metastases, skull metastases, cystic lesions, and LMs.

It was recommended that the CNS and the non-CNS compartments should be evaluated separately. CNS and non-CNS progression should be assessed based on the RANO-BM and RECIST 1.1 criteria, respectively. The definition and assessment of BM and LM survival involve: the overall bio-compartmental progression-free survival (PFS) for local CNS lesions, remote CNS lesions, and extracranial non-CNS lesions; CNS PFS for local and remote CNS lesions; extracranial non-CNS PFS; and CNSlocal PFS only for local CNS lesions [13].

Treatments

For driver gene-positive tumors

A retrospective study found that, patients with EGFR mutations had a higher incidence of LM than those with wild-type EGFR (9.4% vs 1.7%; $P < 0.001$); the time interval from the diagnosis of metastatic lung cancer to the occurrence of LM was 13.3 months [3]. This study also showed that patients receiving tyrosine kinase inhibitor (TKI) therapy had longer overall survival (OS) than those who were not (10 months vs 3.3 months; $P < 0.001$) [3]. A combined regimen of TKI and whole brain radiotherapy (WBRT) failed to achieve further survival benefits. On the other hand, it was also found that the Eastern Cooperative Oncology Group (ECOG) score is a survival indicator of poor prognosis (< 2 vs ≥ 2; HR, 3.657; $P < 0.001$) [3]. Another study also discovered that patients with NSCLC and EGFR mutations had a similar incidence of LM (9%) and a median survival of 3.1 months [14]. At the time of LM diagnosis, patients with an ECOG score of 0–1 showed longer survival than those with a score ≥ 2. Another retrospective study also showed that the use of EGFR-TKI therapy is an independent predictor of increased post-diagnosis survival rates in NSCLC patients with LM and EGFR mutations [4].

Erlotinib and gefitinib are first-generation EGFR-TKIs. The former is able to reach a higher concentration in the CSF (66.9 nM vs. 8.2 nM; $P = 0.0008$) and has a higher penetration rate than the latter (2.8% vs 1.13%) [15]. A retrospective study comprising 25 cases of LM indicated that erlotinib might be more effective than gefitinib in the treatment of LM and that it had a higher cytologic conversion rate in the CSF than the latter (64.3% vs 9.1%; $P = 0.012$) [16]. Another retrospective study compared the therapeutic efficacy of high-dose erlotinib (200 or 300 mg every 2 days, 300 or 450 mg every 3 days, or 600 mg every 4 days) with that of standard-dose erlotinib or gefitinib in patients with EGFR-mutant lung cancer and refractory LM after they had developed resistance against standard-dose erlotinib or gefitinib [17]. The results showed that the two groups had similar median survival (6.2 months for the high-dose group vs 5.9 months for the standard-dose group; $P = 0.94$). According to yet another retrospective study, high-dose EGFR-TKI failed to prolong the survival of LM patients (2.4 months for the high-dose group vs 3.1 months for the standard-dose group; $P = 0.863$) [14]. Despite the use of EGFR-TKI at a standard dose, nine patients with EGFR-mutant NSCLC were still experiencing refractory CNS metastases. A retrospective study on high-dose, pulsatile erlotinib therapy (at the median dose of 1500 mg once a week) revealed that three patients had isolated LM, whereas one had isolated BM, and five had both types of lesions [18]. Among these nine patients (including two with isolated LM), six (67%)

displayed radiological improvement and had a median OS of 12 months. The patients demonstrated satisfactory tolerance to treatments, and no severe toxicity (grade 3 or above) was observed. After pulsatile therapy, the drug concentration in the CSF was 130 nM, which was higher than the IC50 of erlotinib [19].

Afatinib is a second-generation EGFR-TKI. Tamiya *et al* reported the therapeutic efficacy and CSF concentration of afatinib in 11 patients with EGFR-mutant NSCLC and LM. Afatinib had a median penetration rate of 1.65% and a median concentration of 1.4 ng/mL (2.9 nM) in the CSF, which was higher than the previously reported concentration of 1 nM [20]. There was a patient response rate of 27.3%, median OS of 3.8 months, and median PFS of 2 months.

Osimertinib is a third-generation EGFR-TKI. With its excellent efficacy against systemic and CNS metastatic tumors, it is considered a standard regimen for EGFR Thr790Met mutation-positive metastatic NSCLC [21]. Studies have also been conducted on osimertinib as a treatment for LM. In a prospective study, Nanjo *et al* examined the therapeutic efficacy of standard-dose osimertinib (80 mg per day) by observing 13 cases of patients with Thr790Met-positive NSCLC after the treatment failure of standard-dose erlotinib, gefitinib, or afatinib [22]. Among them, five patients were cytologically diagnosed as having LM, whereas eight had suspected LM. The median PFS among all 13 patients was 7.2 months, and the osimertinib penetration rate into the CSF was 2.5%. A study published in New England compared the efficacy of osimertinib with that of the combination chemotherapy of platinum therapy plus pemetrexed in advanced NSCLC; the median PFS of the osimertinib group was significantly longer than that of the platinum–pemetrexed group [10.1 months vs. 4.4 months; HR, 0.30; 95% confidence interval (CI), 0.23–0.41; $P < 0.001$]. The objective response rate (ORR) of osimertinib (71%; 95% CI, 65–76) was significantly better than that of the platinum-pemetrexed group (31%; 95% CI, 24–40; ORR, 5.39; 95% CI, 3.47–8.48; $P < 0.001$). Among 144 patients with CNS metastases, those receiving osimertinib therapy had a longer median PFS than those in the platinum-pemetrexed group (8.5 months vs. 4.2 months; HR, 0.32; 95% CI, 0.21–0.49). The proportion of patients with adverse events of grade 3 or higher was significantly lower with osimertinib (23%) than with the regimen of platinum therapy plus pemetrexed (47%) [21].

Crizotinib is an ATP-competitive inhibitor against ALK/MET/ROS1. It is also the first targeted drug for ALK-positive NSCLC approved by the U.S. Food and Drug Administration. Despite its low penetration rate into the CNS, studies have shown that it can better control CNS diseases than standard chemotherapy [23–24]. Regardless, the CNS is a common site of cancer recurrence in patients who have received crizotinib therapy. There are very few reports about its efficacy against LM.

Ceritinib is a second-generation ALK/ROS1 inhibitor that is more effective than crizotinib. It has higher permeability across the blood-brain barrier and is used for treatment after the development of crizotinib resistance in patients. After treatment failure of standard-dose crizotinib and WBRT in ALK-positive NSCLC patients, the sequential therapy of administering pulse-dose crizotinib (500 mg per day) followed by standard-dose ceritinib (750 mg per day) was found to be able to keep BMs (LMs) under control [25]. Another case report indicated that ceritinib was able to control BM and LM for over 5 months among ALK-positive NSCLC patients receiving chemotherapy and crizotinib therapy [26].

For NSCLC patients carrying EGFR mutations, the response rate to EGFR-TKI therapy for BM (gefitinib, erlotinib, and afatinib) was up to 60%–80%, whereas the complete response rate was up to 40%. The median OS was 15–20 months, and the PFS for patients with intracranial lesions was 6.6–11.7 months, both of which were significantly longer than those of patients with wild-type EGFR tumors.

Surgical resection, stereotactic radiosurgery (SRS), and WBRT have long been the main treatment methods for BM. Recently, a phase II clinical trial reported that using erlotinib alone to treat BM patients yielded a median OS of 15.9–22.9 months and a median PFS of 5.8–14.5 months; the ORR of the patients was 55%–89% [27].

Although many phase II clinical trials studied the efficacy of early application of EGFR-TKI therapy in BM treatment, none of them have compared the efficacy of using TKI before radiotherapy and using radiotherapy before TKI. Therefore, William *et al* conducted a multi-institutional analysis to determine the optimal management of patients with EGFR-mutant NSCLC who had developed brain metastases and had not received EGFR-TKI therapy yet. The conclusion was that postponing BM radiotherapy would lower the patients' OS and that SRS followed by EGFR-TKI could result in the longest OS [27]. Another study demonstrated that the WBRT of patients with EGFR mutations or ALK-positive NSCLC and BM could be safely postponed using highly effective targeted therapy, in order to minimize toxic effects that will decrease the patients' quality of life.

The time from the initial diagnosis to the onset of LM ranges from 7 to 17 months [28–29], accompanied with a generally poor prognosis and a median OS of approximately 3–6 months [29–30]. Before the introduction of EGFR-TKI therapy, the treatment regimen for LM included intrathecal chemotherapy (ITC), WBRT, and ventriculoperitoneal (VP) shunting; but the therapeutic efficacy remained poor [31]. A retrospective study reported the treatment results and prognostic factors of NSCLC LM

patients. In a large-scale retrospective study on NSCLC patients with cytologically diagnosed LM, a few favorable prognostic factors were brought to attention, including patients having received WBRT, ITC, EGFR-TKI, and VP shunt; on the other hand, unfavorable prognostic factors included low PFS score, high CSF protein level, and high CSF white cell count, all of which hinted at a heavier disease burden. Interestingly, the median OS of patients receiving traditional treatment was merely 14 weeks, while the median OS of patients receiving EGFR-TKI therapy was 38 weeks [29]. It was also observed in other retrospective studies that patients receiving EGFR-TKI therapy had a longer OS [32]. However, it is still unclear whether such changes in OS were caused by EGFR mutation status, the use of EGFR-TKI therapy, or both. It is worth noting that most of these small-scale studies selected East Asian patients with a higher EGFR mutation incidence as their main research targets.

Overall, the sources of data related to LM treatment were restricted to single-institutional retrospective studies. Favorable prognostic factors were associated with lower disease burden (such as low intracranial pressure and low white cell count in the CSF). In patients receiving EGFR-TKI therapy, better physical strength and prolonged survival were observed [33].

For driver gene-negative tumors

Fenske *et al* summarized the median OS of NSCLC BM patients treated by different methods across seven countries. In the U.S., NSCLC BM patients treated with systemic chemotherapy had the longest median OS – 11.8 months – compared with those treated with other methods. Yet, in Japan and Italy, patients treated with radiotherapy had a median OS of 13.4 months and 10.5 months respectively, compared with those receiving systemic therapy and surgery. In three countries, surgery resulted in the longest OS – 13.2 months in France, 6.05 months in the U.K., and 5 months in Spain. When the treatment method was taken out of consideration, patients in Japan had the longest median OS of 13.1 months, followed by those in the U.S. and Italy, both of which had a median OS of 10 months. The median OS was 8 months in the U.K., 6.7 months in France, and 5 months in Spain. The German studies did not report the median OS of patients. The U.S. and Japan had a higher median OS than the countries in the European Union. When nationality was put aside, radiotherapy resulted in the longest median OS of 10 months, followed by systemic chemotherapy and surgery, which led to a median OS of 9.15 months and 8.5 months respectively [34].

Anti-angiogenic therapy

Bevacizumab is a recombinant humanized monoclonal antibody. It can selectively bind with VEGF and prevent it from reacting with its receptors. The combined use of bevacizumab and platinum-containing chemotherapy has been authorized as the first-line treatment for advanced, metastatic, or recurrent and non-squamous NSCLC.

The phase II prospective, non-comparative BRAIN study (NCT00800202) examined asymptomatic and untreated patients with stage IV non-squamous NSCLC and BM who received first-line bevacizumab (15 mg/kg) plus carboplatin (area under the curve = 6) and paclitaxel (200 mg/m^2) every 3 weeks (B + CP) or second-line bevacizumab plus erlotinib (150 mg/d; B + E) therapy. The safety and efficacy of using bevacizumab to treat asymptomatic and untreated NSCLC BM patients were observed. The results showed that, in the first-line B + CP group (n = 67), the 6-month PFS rate was 56.5%, whereas the median PFS was 6.7 months, and the median OS was 16.0 months. The investigator-assessed ORR was 62.7%; the intracranial lesion incidence was 61.2%, and the extracranial lesion incidence was 64.2%. Due to the low enrollment rate (n = 24), the efficacy results for the second-line B + E group were merely exploratory – the 6-month PFS rate was 57.2%, whereas the median PFS was 6.3 months, and the median OS was 12.0 months; the ORR was 12.5%. The adverse events were comparable to those in previous bevacizumab trials. Grade 1 intracranial hemorrhage occurred and was resolved with no sequelae. This study verified the efficacy and safety of using first-line bevacizumab with paclitaxel and carboplatin for treating asymptomatic and untreated NSCLC BM patients [35].

Traditional chemotherapy

A post-hoc analysis was conducted on the BM patients observed in a large-scale, prospective, and observational study on the first-line treatment of NSCLC – the European FRAME study. It aimed to describe the baseline characteristics of NSCLC BM patients, understand their first-line treatment, and report real-life treatment outcomes. BM patients and the overall cohort had a median OS of 7.2 months and 10.3 months respectively; the median PFS was 3.6 months and 5.6 months respectively, whereas the 1-year survival rates were 30% and 45% respectively. Patients treated with pemetrexed plus platinum had a median OS of 9.3 months (95% CI, 6.2–11.9), whereas those treated with gemcitabine plus platinum had a median OS of 5.6 months (95% CI, 4.1–8.4). The results were in line with those of the recently published retrospective analysis on a database of 1833 cases of NSCLC BM, which reported a median OS of 7.0 months (95% CI, 6.5–7.5) while highlighting the significant heterogeneity in the results. On the other hand, a retrospective cohort study on all new lung cancer cases in institutions in Canada between July 2005 and June 2007 showed that the median OS among 91 NSCLC BM patients was 7.8 months [36].

Despite some recent improvements in radiotherapy

technologies, such as surgical resection of single brain lesions and SRS for oligometastases, WBRT remains the fundamental treatment for BM, whereas systemic chemotherapy remains the basic treatment for disseminated NSCLC. Recent data revealed that pemetrexed plus platinum-based chemotherapy could be a sensible option for asymptomatic BM patients and could prevent such patients from receiving early radiotherapy to the head. The pemetrexed cohort was the largest treatment group in the study on BM patients and had a 1-year survival rate of 39% (95% CI, 29–48). Due to the possibility of selection bias, the results were not directly comparable between cohorts. Therefore, these descriptive data should be interpreted with caution. The OS reported in that study could merely represent some NSCLC BM patients receiving platinum-containing combination chemotherapy [36].

Immunotherapy

Check-point inhibitors that are currently available include atezolizumab, nivolumab, and pembrolizumab. The sample of CNS metastasis patients treated with single-agent immune-oncology (IO) therapy is small, and treatment is restricted by tight constraints. Based on the existing data, atezolizumab is, at present, the only IO drug observed to have evident survival benefits to BM patients; nivolumab has been observed to have the same therapeutic efficacy in both CNS and non-CNS metastasis patients. A prospective, small-sample study preliminarily confirmed that pembrolizumab is effective in treating patients with CNS metastasis.

Conclusion

BMs (LMs) should be scored and rated; a recommended tool for doing so is the GPA. The three key elements of LM diagnosis include clinical evaluation of CNS functions, imaging manifestations, and CSF cytological examination. The genetic profile of CSF mutations in LM is different from that of the primary tumor and blood-based circulating tumor DNA; mutated genes can be detected in the CSF. Hence, next-generation sequencing of the CSF is recommended for eligible individuals.

Clinical trials should include BM patients as much as possible to ensure the universality of the trial results; a combination of RECIST 1.1 and RANO-BM was recommended as the standard for efficacy assessment. The endpoints of clinical trials should include indicators of efficacy assessment for BM and LM; both separate and comprehensive assessments should be performed.

TKI was recommended as the top treatment option for NSCLC with BMs (LMs) and positivity for EGFR, ALK, or any other driver genes; as for recurrent LM, high-dose, pulsatile TKI therapy can be considered (gefitinib 500–1000 mg orally every other day for 14 days or erlotinib 1500 mg orally once a week +/– bevacizumab 10 mg intravenously once every 2 weeks); clinical trials on sequential therapy of TKIs or combination therapy of TKIs and WBRT for multiple BM were also recommended. Radiotherapy, systemic chemotherapy, and ITC remain as the main treatment methods for driver gene-negative multiple BMs (LMs). The optimal chemotherapy regimen has yet to be determined, but pemetrexed appears to offer better survival benefits to patients with adenocarcinoma BM. Anti-angiogenic therapy is shown to have promising prospects due to its anti-BM (LM) efficacy. The therapeutic activity of check-point inhibitors has been demonstrated in small-scale trials.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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REVIEW ARTICLE

Long noncoding RNAs as diagnostic biomarkers associated with cancer phenotypes

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Abstract

Increasing evidence suggests that long noncoding RNAs (lncRNAs) play vital roles in the transformation and maintenance of cancer phenotypes and have important clinical implications. These lncRNAs control important aspects of tumor biology, including proliferation, angiogenesis, metastasis, and the microenvironment by regulating RNA and protein interactions or through their ability to base pair with RNA and DNA. In this study, we review the mechanism of the function of lncRNAs in cancer and their diagnostic roles in cancer phenotypes, which make them attractive as non-invasive biomarkers from body fluid samples for different types of cancer.

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Carcinogenesis is regarded to comprise genetic or epigenetic alterations that are based on two constituent processes, the continuous acquisition of heritable genetic variation in individual cells by random mutation and natural selection acting on the resultant phenotypic diversity. Furthermore, several important studies suggest that cancer is a disease of the genome, which comprises heterogeneous clonal expansions driven by the accumulation of mutations that are preferentially selected by the tumor microenvironment [1]. Many of these mutation sites overlap regions of the genome that lack protein-coding capacity. These abnormalities have an impact on noncoding RNA molecules, which display altered expression and disrupted functions in terms of regulation of their targets.

Approximately, 19,000–20,000 human protein-coding genes have been estimated to be present in the human genome. Protein-coding sequences make up only a small fraction of the genome (no more than 2%), and a large number of sequences are associated with non-coding RNA moleculars. Among noncoding RNAs, long noncoding RNAs (lncRNAs), with a length > 200 bp, have increasingly been recognized to play vital roles in tumor biology, representing a new focus in the study of cancer. Emerging technologies are expanding investigators' abilities to functionally annotate cancer-associated lncRNAs. Importantly, cancer-specific expression of certain lncRNAs has provided the necessary impetus to lncRNA research and highlighted the importance of these molecular modulators, which has been verified in the pathological states of carcinogenesis [2]. With regard to their role in cancer, lncRNAs show tissue-specific expression in a specifically regulated manner, in correlation with distinct gene sets that influence cell cycle regulation, survival, immune response, or pluripotency, among other functions, which determine the transformed phenotype of the cancer cells. In fact, lncRNAs play an important role in regulating gene expression at various levels, including chromatin, modification, transcription, and post-transcriptional processing [3–4]. Conversely, several lncRNAs are also transcriptionally regulated by key tumor suppressors or oncogenes. For example, lncRNA p21 is mediated by p53-dependent transcriptional responses, which affect the expression of hundreds of gene targets enriched for the gene sets normally repressed by p53 [5]. Zheng et al. also demonstrated that the oncogenic transcription factor cMyc is partly responsible for lncRNA expression [6]. The regulatory function occurs in many types of cancer that are involved in the specific genomic context of divergent transcription. In particular, recognition of the roles lncRNAs has revealed new diagnostic and therapeutic targets. LncRNAs appear to be more structured and stable than mRNA transcripts, the

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measurement of lncRNAs as free nucleic acids could trace cancer metastases or circulating cancer cells in body fluids, such as blood and urine. For example, the overexpression of the lncRNA *HOTAIR* promotes the metastasis of breast cancer cells by epigenetically silencing the developmentally important genes in the *HOXD* cluster [7–8]. In addition, panels of lncRNAs have already been put to good use in clinically approved tests for bladder, prostate, and non-small lung cancer [9–11]. LncRNAs are thus functional transcripts that contribute to the hallmarks of cancer. Further research into the relationship between cancer and the roles of lncRNAs will be crucial to understand and realize their therapeutic potential.

In this work, we provide an overview of the current state of lncRNA biomarker identification in cancer phenotypes linked to invasion/metastasis, angiogenesis, genome instability, and tumor-promoting inflammation.

Molecular background of lncRNAs

The catalog of lncRNAs has gradually increased in recent years. An lncRNA can be placed into one of approximately five broad categories, including sense, antisense, bidirectional, intronic, and intergenic lncRNAs [12]. According to LNCipedia2.0, the latest version of the lncRNA database, there are already 32,183 human annotated lncRNAs; however, few lncRNAs have been functionally validated [13]. Therefore, it should be elucidated theoretically as to whether most of these lncRNAs result from transcriptional noise. To answer this question, a study by Ponjavic *et al* analyzed 3122 long and full-length noncoding RNAs that exhibited signatures of functionality that are more usually associated with protein-coding genes [14]. Furthermore, Dinger *et al* have constructed a database that should provide the expression status and other valuable resources for mammalian lncRNAs [15]. Most recently, TANRIC, an open-access web resource, providing interactive exploration of lncRNAs in cancer, was constructed to characterize the expression profiles of lncRNAs in large patient cohorts of 20 cancer types, based on the Cancer Genome Atlas (TCGA) and independent datasets [16].

LncRNAs are much longer than microRNAs, and thus have a complex secondary structure, which endows lncRNAs with the ability to bind protein, RNA, and/or DNA partners. Thus, they can have several regulatory capacities, for example as activators, decoys, guides, or scaffolds for their interacting proteins, including behaving as transcription factors and histone modifiers. In the present review, we summarize the mechanism of lncRNAs' regulatory cellular processes that rely on interactions with cellular macromolecules.

Chromatin-bound lncRNAs

Chromatin remodeling was one of the first identified functions of lncRNAs. An lncRNA is generated by antisense transcription from the fibroblast growth factor receptor 2 (*FGFR2*) locus, which promotes cell-specific alternative splicing via modulation of the chromatin signature [17]. Meanwhile, prostate cancer associated 3 (*PCA3*) also is an antisense intronic lncRNA that controls the expression level of prostate cancer suppressor prune homolog 2 (*PRUNE2*) via formation of a double-stranded complex, after which the adenosine deaminase, RNA specific (ADAR)-mediated RNA editing mechanism downregulates the expression of its target gene [18]. The X-linked lncRNA Firre, helps to position the inactive X chromosome near the nucleolus and preserves one of its main epigenetic features [19]. Meanwhile, during X chromosome inactivation, lncRNA RepA can silence the expression of polycomb repressive complex 2 (*PRC2*), the mechanism of which involves the interaction between histone methyltransferase and the lncRNA 18. Conversely, lncRNAs can organize chromatin domains to coordinate long-range gene activation, such as in the case of HOTTIP and CCAT1-L, which regulate chromosome looping in their proximity to deposit activating H3K4me3 marks on gene promoters [20–22]. Importantly, recent work reported that lncRNA recruitment to distant promoters and enhancers functionally modulates cancer transcriptional programs. Such RNAs make an important contribution to the maintenance of certain transcription factors (TFs) at gene regulatory elements, which produces a positive-feedback loop that contributes to the stability of gene expression programs [23].

LncRNA and DNA methylation cooperate in the epigenetic regulation of the cancer genome

Epigenetic changes in malignant diseases have been described, such as DNA hypermethylation on CpGs islands or genetic control physical domains at several tumor-suppressor genes, oncogenes, and DNA repair genes. In addition, hypermethylation is associated with aberrant post-translational modifications on histone tails, as well as lncRNAs patterns and their levels of expression. Important evidence is provided by the lncRNA HOTAIR and its functional histone mark H3K27me3, which is directly associated to the expression level of PRC2 [24]. This function is based on a fundamental role of lncRNAs, as molecular guides or scaffolds that cooperate with methylation signals, acting as a decoy mechanism to control regional epigenetic changes throughout the human cancer genome.

LncRNAs interact with target proteins as scaffolds to modify their stability

Interactions between lncRNAs and proteins have significant effects. Interestingly, many lncRNAs exert their unique activities in cancer cells. Using RNA immunoprecipitation, two prostate-specific lncRNAs, PCGEM1 and PRNCR1, were found to associate with the androgen receptor in prostate cancer cells and cause ligand-independent activation of cell proliferation [25]. Similarly, CTBP1-AS and CCTA2 interact with TFs to modify their activity [26–27]. Furthermore, the lncRNA HOTAIR serves as a scaffold that forms a complex with Hepatitis B virus X-interacting protein (HBXIP) and lysine demethylase 1A (LSD1) to activate transcription of c-myc targeted genes [28].

LncRNAs serve as regulators of mRNA expression

Emerging evidence supports the view that lncRNAs play vital roles in the control of mRNA stability, splicing, and translation. Previously, Tripathi *et al* demonstrated that MALAT1 regulates alternative splicing by modulating the phosphorylation of SR splicing factor *in vitro* [29]. Furthermore, the lncRNA, antisense to zinc finger E-box binding homeobox 2 (*ZEB2*), regulates the expression of its target gene by impaired splicing of the internal ribosome entry site contained in an intron during epithelial–mesenchymal transition (EMT) [30]. In addition to alternative splicing, MALAT1 can also interact with pre-mRNA that directs itself to localize at the proximal chromatin region of transcriptionally active genes [31]. In addition, some lncRNAs form DNA-RNA triplexes that regulate the expression of oncogenes, such as sphingosine kinase 1 (*SPHK1*) and transforming growth factor beta (*TGFB*) via antisense orientation to their promoters [32–33].

Taken together, research has shown that lncRNAs perform functional interactions or combinations with DNA, RNA, and protein, which suggest that lncRNAs served as a multifunctional tool in several biological processes. Next, we discuss the relationship between lncRNAs and the phenotype of carcinogenesis, to further determine their contribution to cancer hallmarks.

The contribution of lncRNAs to cancer hallmarks

Hanahan and Weinberg defined the hallmarks of cancer as acquired functional capabilities that allow cancer cells to survive, proliferation, and metastasis in 2011 [34]. Two prominent characteristics of tumorigenesis were emphasized in that paper: The development of genomic instability and the tumor microenvironment. Recently, lncRNAs have been identified as, key molecular players in proliferation, viability, angiogenesis, and metastasis [35–36]. In addition, other new signatures of lncRNAs are emerging.

Modulating proliferative signaling

Cancer cells, by deregulating proliferative signals, become masters of their own proliferative destinies. Numerous studies have demonstrated that cancer-related changes in lncRNA expression could promote cancer growth, mainly by acquiring pro-growth signals and evading the growth suppressive signals.

Multiple lncRNAs are involved in the regulation of critical cell cycle regulators, such as cyclins, cyclin dependent kinases (CDKs), and p53 [37]. For example, the cyclin D1 lncRNA specifically binds with an RNA-binding protein, TLS (translocated in liposarcoma), and exerts transcriptional repression through histone acetyltransferase inhibitory activity [38]. The lncRNA ANRIL binds to and recruits PRC2 to repress the expression of p15 (cyclin dependent kinase inhibitor 2B (CDKN2B)) [39].

The lncRNA PANDA confines cells to their existing proliferative state by repressing the transcription of senescence-promoting genes, which represents a stable cell cycle arrest that limits the proliferation of pre-cancerous cells [40]. In a DNA damage-dependent manner, lncRNA Gadd7 binds to the TAR DNA-binding protein, and further modulates the expression of CDK6 at the post-transcriptional level by its altering mRNA stability [41]. Meanwhile, the expression of lncRNA HEIH in HBV-hepatocellular carcinoma is associated with recurrence and is an independent prognostic marker for survival, the mechanism of which involves G0/G1 arrest [42]. Importantly, MALAT1, an mRNA splicing mediator, is upregulated in several human cancers and contributes to cancer cell proliferation [29, 37]. The underlying mechanism is that MALAT1 promotes cellular proliferation by modulating the pre-mRNA processing of cell cycle-regulated transcription factors, such as Mybl2, an oncogenic transcription factor involved in G2/M progression [43]. In addition, Zhang *et al* demonstrated that p53 is significantly downregulated by the lncRNA ROR, which suppresses p53 translation through direct interaction with a heterogeneous nuclear ribonucleoprotein [44]. Furthermore, Myc transcription is activated in *cis* by the colon cancer-associated lncRNA CCAT1, which facilitates the long-range interaction between Myc and an enhancer element [45]. Inversely, Myc also targets numerous lncRNAs for transcriptional regulation [6], which in turn regulates cell-cycle

progression.

Inducing angiogenesis

Normally, as part of physiological processes such as wound healing and female reproductive cycling, angiogenesis is turned on, but only transiently. In contrast, during tumor progression, an "angiogenesis switch" is almost always activated and remains on [34]. The best-known angiogenic switch is vascular endothelial growth factor (VEGF). Recently, transcription of *VEGF* was identified to be modulated by multiple lncRNAs. LncRNA PVT1 is upregulated and is significantly associated with high-microvessel density and poor prognosis in gastric cancer. The mechanism of PVT1-mediated angiogenesis involves in evoking the signal transducer and activator of transcription 3 (STAT3)/VEGF-A signaling axis [46]. Similarly, lncRNAs MVIH, MIAT, and SUMO1P3 have also been reported to promote the expression of *VEGF* [47–49]. Furthermore, lncRNA GATA6-AS is upregulated in endothelial cells during hypoxia. A compelling body of evidence indicates that GATA6-AS interacts with the epigenetic regulator lysyl oxidase like 2 (LOXL2) to regulate endothelial gene expression via changes in histone methylation [50].

Influencing invasion and metastasis

The multistep process of invasion and metastasis has been conceived as a sequence of discrete steps, often termed the invasion-metastasis cascade [51], the beginning of which is EMT. During this developmental regulatory program, the transformed epithelial cells can acquire the ability to invade, resist apoptosis, and disseminate [52]. With recent advances in transcriptome analysis technologies (such as RNA-seq), emerging evidence shows that lncRNAs that are differentially expressed in tumors correlate their metastatic properties, especially EMT. Some lncRNAs, such as ATB, stabilize interleukin 11 (*IL11*) mRNA, and elevated IL-11 secretion, which induces EMT and invasion [53]. Moreover, ATB also serves as an independent prognostic marker in gastric [54] and colorectal cancer [55]. Kim *et al* reported that there is a long-range interaction and correlation between a Myc enhancer and the promoter of the lncRNA CARLo-5 [45], which has some effects on EMT, and predicts outcome in patients with hepatocellular carcinoma [56]. In contrast, the lncRNA Gas5 was reported to be a negative regulator of survival and proliferation of several cancers [57]. Low expression of Gas5 correlates with poor prognosis of breast cancer and head and neck squamous cell carcinoma [58]. In line with this, Zhao *et al* indicated that Gas5 suppresses the migration of glioma cells by downregulating the expression of microRNA miR-222 [59]. With the growing number of studies on the association of lncRNAs with metastatic properties, the potential of these types of lncRNAs as therapeutic targets and prognostic markers will be a topic of active research.

Table 1 Example biomarkers of cancer-associated lncRNAs

LncRNA	Description	Functions in tumor cells	Involved Mechanism
ABT [53–55]	Activated by TGF-beta	Metastasis ↑	RNA-RNA activation / translational regulation
ANRIL [39, 70–75]	Antisense lncRNA in the INK4 Locus (CDKN2B-AS)	Proliferation ↑ , Metastasis ↑	Chromatin remodeling
BANCR [76–78]	BRAF regulated lncRNA	Proliferation ↑ , Metastasis ↑	Transcriptional activation
BCAR4 [79–82]	Breast cancer antiestrogen resistance 4	Proliferation ↑ , Metastasis ↑	Binding to transcription factor / Transcriptional activation
CARLo-5 [45, 56]	Active regulator region of lncRNA	Proliferation ↑ , Metastasis ↑	RNA-DNA interaction /Binding to enhancer region of MYC
CCAT1/ CCAT2 [20, 26]	Colon cancer specific transcript 1/2	Proliferation ↑ , Metastasis ↑	Chromatin remodeling /Transcriptional activation
DINO [83]	Damage Induced lncRNA via p53	Proliferation ↓	Activation of p53 target genes in response to DNA damage
MVIH [49]	LncRNA associated with microvascular invasion	Angiogenesis ↑ ↑	Unknown
PVT1 [46]	STAT3-responsive lncRNA	Angiogenesis ↑	The binding of PVT1 activated the STAT3 signaling pathway
PACER [66]	P50-associated COX-2 extragenic RNA	Proliferation ↑ , Metastasis ↑ , Inflammation ↑	Activation-competent NF-kappa B p65/p50 dimers

Regulating the tumor-associated inflammatory response

As is well known, the relationship between inflammation and carcinogenesis is analogous to that between "fuel and fire" [60]. Inflammation is demonstrably capable of fosteringthe development of incipient neoplasias into cancers. It is increasingly clear that lncRNAs control the key aspects of immunity such as production of inflammatory mediators, differentiation and immune cell recruitment through regulating protein-protein or RNA-DNA interactions [61]. Recently, the roles of lncRNAs in controlling NF-κBsignaling have attracted much attention [62]. Lethe, a pseudogene lncRNA, is selectively induced by proinflammatorycytokines via NF-κB, and functions in negative feedback signaling to NF-κB [63]. During the activation of macrophages, lncRNA Tnfaip3 acts as a coregulator of NF-κB to modulate inflammatory gene transcription via epigenetic chromatin remodeling [64]. In addition, NKLIA is upregulated in breast cancer cells by NF-κB, binds to NF-κB/IκB, and directly masks of phosphorylation motifs of IκB [65]. COX-2, an important oncogenehas been linked to development, progression, and outcome of several types of human cancer.Krawczyk *et al*. identified the COX-2-lncRNA,PACER occludes NF-κB subunit p50, potentially facilitating interaction with activation competent NF-κB p65/p50 dimers [66]. Furthermore, lncRNA TCF7 is required for liver cell stem cell self-renewal and tumor proliferation. Mechanistically, TCF7 recruits the SWI/SNF complex to the promoter of TCF7 to regulate its expression, leading to activation of Wnt signaling [67]. Interestingly, Zhou *et al*. validated immune associated lncRNAs signature, which is significantly linked to the clinical molecular subtypes and prognosis in diffuse large B cell lymphoma [68].

Conclusion

Overall, increasing evidence suggests that lncRNAs play vital roles in the transformation and maintenance of cancer phenotypes, and have important clinical implications. Actually, the function and characteristics of lncRNAs have made them a well suitedcandidate for cancer molecular diagnosis (summarized together in Table 1). Importantly, lncRNAs show more tissue specificity compared to protein-coding mRNAs and miRNA[69], making them attractive in the search of novel non-invasive diagnosticbiomarkers from body fluid samples. In the future, more studies will be performed to evaluate the diagnostic value of lncRNAs in different types of cancer.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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ORIGINAL ARTICLE

Treatment and survival status of patients with *EGFR* mutation-positive stage IV lung adenocarcinoma: five-year follow-up results in the Ordos Area of Inner Mongolia, China*

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Abstract

Objective We aimed to determine the epidermal growth factor receptor (*EGFR*) mutation status and treatment survival of patients with stage IV lung adenocarcinoma living in the Ordos area of Inner Mongolia, China.

Methods *EGFR* testing and first-line tyrosine kinase inhibitor (TKI) treatment rates of patients with stage IV lung adenocarcinoma were analyzed from June 2012 to June 2016. Kaplan-Meier survival curves were constructed to compare patients who received different treatment strategies and those harboring different *EGFR* mutation statuses.

Results *EGFR* testing and mutation rates were 65.60% and 52.90%, respectively, and improved continuously from June 2012 to June 2016. Among patients with *EGFR* mutations, 38.9% had *EGFR* 19 del, 48.2% had L858R, 4.2% had co-existing mutations in exons 19 and 21, and 8.4% had uncommon mutations. The median overall survival (OS) was 29.5, 26.5, and 16.0 months for patients receiving both TKI and chemotherapy, TKI alone, and chemotherapy alone, respectively (P = 0.047). The OS was 26.5 and 30.0 months for patients harboring *EGFR* 19 del and L858R mutations, respectively (P = 0.096).

Conclusion The high OS rates of stage IV lung adenocarcinoma patients living in the Ordos area may be attributed to continuous improvements in *EGFR* testing and first-line TKI treatment rates. In the era of TKIs, chemotherapy for increasing OS times should be emphasized.

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Key words: epidermal growth factor receptor (EGFR); tyrosine kinase inhibitor (TKI); minority areas

Lung cancer is the leading cause of cancer-related mortality worldwide, and non-small cell lung cancer (NSCLC) accounts for 88% of lung cancer cases [1]. In China, lung cancer accounts for 25.24% of deaths among the 10 cancer types most commonly associated with mortality in cancer registration areas in 2009 [2]. In recent years, the percentage of patients with adenocarcinomas has increased significantly such that it has now become the most common cancer histologically [1].

Guidelines for NSCLC management strongly recommend testing for epidermal growth factor receptor (*EGFR*) gene mutations and administering tyrosine kinase inhibitors (TKIs) as first-line treatment in patients harboring such mutations because of the reported improvements in life quality and overall survival (OS) [3]. Previous national surveys showed that the rate of *EGFR* mutation testing was only 9.6% in China because of the limited access to relevant technology [4]. A multicenter survey from 12 tertiary hospitals showed an increased gene aberration testing rate of 71.4% compared with those reported in national surveys, although these hospitals were all affiliated with the medical universities in China, which reported high rates of lung cancer diagnoses and treatments [5]. Moreover, only 53.5% of *EGFR* mutation-

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positive patients received *EGFR* TKIs as first-line treatment at Guangdong Lung Cancer Institute, China [6]. Therefore, it is particularly important to continuously improve the *EGFR* testing and first-line TKI treatment rates in patients with stage IV lung adenocarcinoma.

The retrospective study aimed to identify the extent to which national treatment guidelines were adopted to customize care for lung adenocarcinoma patients living in the Ordos area of Inner Mongolia, China, between June 2012 and June 2017.

Patients and methods

Study population

In this retrospective observational survey, clinical data of patients with advanced lung adenocarcinoma were obtained from an electronic database at Department of Medical Oncology, Ordos Central Hospital, China, from June 2012 to June 2017. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ordos Central Hospital Committee on Human Research. All patients provided a written informed consent to participate in the study.

Data collection

The electronic database information included patient number, age, sex, ethnicity, smoking history, histological grade, genetic status, metastasis sites, and treatment. Smoking history was self-reported. "Never-smokers" were defined as patients who had smoked < 100 cigarettes over their lifetime. All patients had stage IV lung adenocarcinoma. Treatments were described as those administered since the diagnosis of stage IV lung adenocarcinoma and included chemotherapy, TKI therapy, and radiotherapy.

Statistical analysis

Data were presented as medians or numbers (percentages). We analyzed continuous changes in the *EGFR* exon 19 or 21 testing rate and first-line TKI treatment rate from June 2012 to June 2016. OS analysis was conducted in patients harboring *EGFR* 19 del only, L858R mutation only, and co-existing *EGFR* mutations in exons 19 and 21. OS was measured from the date of lung cancer diagnosis to death of any cause from June 2012 to June 2017. Patients were categorized into three groups based on the management modality they received: TKI and chemotherapy, TKI alone, and chemotherapy alone. Kaplan-Meier survival curves were constructed to compare the differences between groups. All statistical tests were two-sided P tests. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS 19.0 software (IBM SPSS, Armonk, NY, USA).

Results

EGFR mutation status

Data of 288 patients with pathology-confirmed stage IV lung adenocarcinoma were included in the electronic database at Department of Medical Oncology, Ordos Central Hospital, China, between June 2012 and June 2017. Of these, 189 (65.60%) patients underwent *EGFR* testing, and testing specimens included biopsy tissues (140/189, 74.07%), pleural fluid samples (23/189, 12.17%), and blood (26/189, 13.76%). The detection of *EGFR* mutation was mainly performed using the amplification refractory mutation system [7], except for six patients who underwent *EGFR* sequencing between June 2012 and December 2013. Among the 189 patients who had *EGFR* testing, 100 (52.90%) had mutations in exon 18, 19, 20, or 21. Of the 100 *EGFR* mutation-positive patients, we excluded five whose mutations were not accurately reported. Among the remaining patients, 38.9% (37/95) harbored *EGFR* 19 del, 48.2% (46/95) had L858R, 4.2% (4/95) had co-existing *EGFR* mutations in exons 19 and 21, and 8.4% (8/95) harbored an uncommon mutation.

EGFR testing and fist-line TKI treatment rate

The *EGFR* testing rate improved continuously from June 2012 to June 2016 (Fig. 1). Additionally, the first-line TKI treatment rate of patients harboring *EGFR* mutations also improved continuously (Fig. 2).

EGFR mutation-positive patient treatment and survival status in the real world

From June 2012 to June 2017, 83.0% (83/100) of patients harboring *EGFR* 19 del or L858R mutations received first-line chemotherapy or TKI treatment; patients with co-existing *EGFR* mutations in exons 19 and 21 were excluded from the treatment analysis. The primary end point of the retrospective study was OS. Patients were categorized into three groups according to the management modality that they received (Fig. 3): group 1 included patients who received first-line TKI with second-line chemotherapy, first-line chemotherapy with second-line TKI, or first-line chemotherapy maintained by TKI (30.1%, 25/83); group 2 included patients who received TKI alone (63.9%, 53/83); and group 3 included who received chemotherapy alone (6.0%, 5/83). The median OS of the three groups was 29.5, 26.5, and 16.0 months, respectively ($P = 0.047$).

Comparison of OS for *EGFR* mutations in exons 19 and 21

Among the 53 patients harboring *EGFR* mutations who received TKI treatment alone, 88.7% (47/53) had *EGFR* 19 del or L858R mutations and 11.3% (6/53) had an uncommon *EGFR* mutation. Among the 47 patients

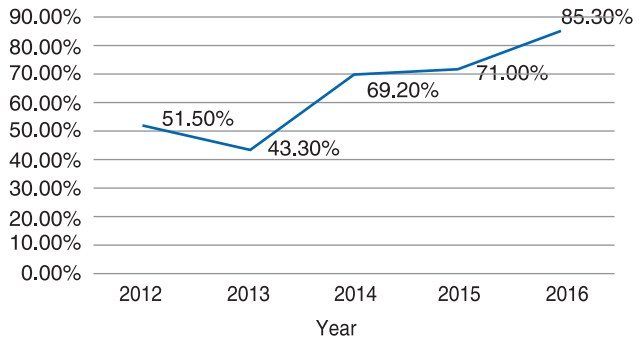

Fig. 1 *EGFR* gene testing rates from June 2012 to June 2016

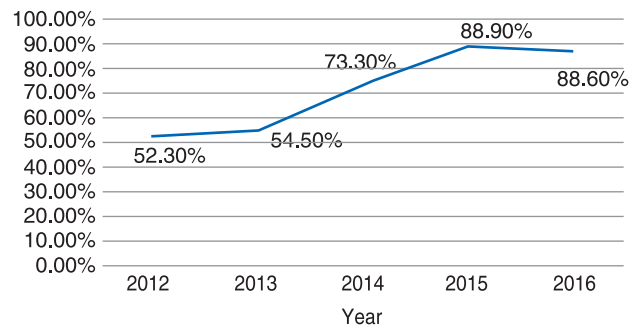

Fig. 2 First-line TKI treatment rates from June 2012 to June 2016

with *EGFR* mutations in exons 19 and 21, 11 had brain metastasis at diagnosis. A comparison of the remaining (36/47, 76.6%) patients without brain metastasis (Fig. 4) revealed an OS of 26.5 months and 30.0 months in those harboring 19 del or L858R mutations ($P = 0.096$). Patients harboring *EGFR* 19 del or L858R mutations without brain metastasis received first-generation TKI without chemotherapy during the entire disease process, although six patients received third-generation treatment after the first-generation TKI therapy failed.

Discussion

In this survey, we retrospectively analyzed the changing trends of *EGFR* testing and first-line TKI treatment rate in patients with stage IV lung adenocarcinoma living in the Ordos area of Inner Mongolia in the last 5 years. Both the *EGFR* testing rate and first-line TKI treatment rate increased sharply from June 2012 to June 2016. To some extent, this finding indicated the continuous advancement in clinical practice in accordance with the guidelines for the management of NSCLC patients harboring *EGFR* mutations in the minority areas of western China [3]. Nearly two-thirds of patients with stage IV lung adenocarcinoma had testing for *EGFR* aberration, and 52.90% of those tested had mutations. This *EGFR* mutation rate was similar to that (59.70%) observed in an

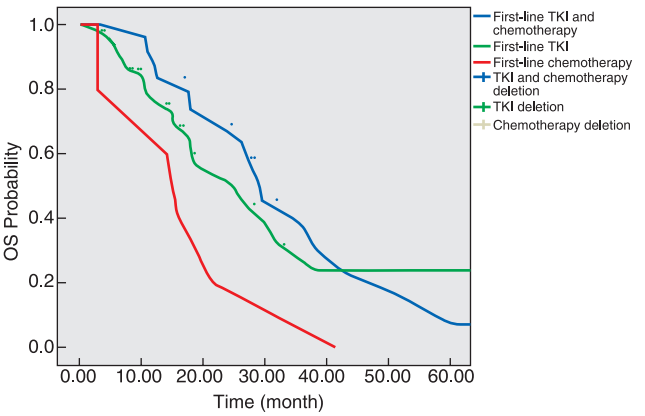

Fig. 3 OS comparison for patients who received different treatment strategies

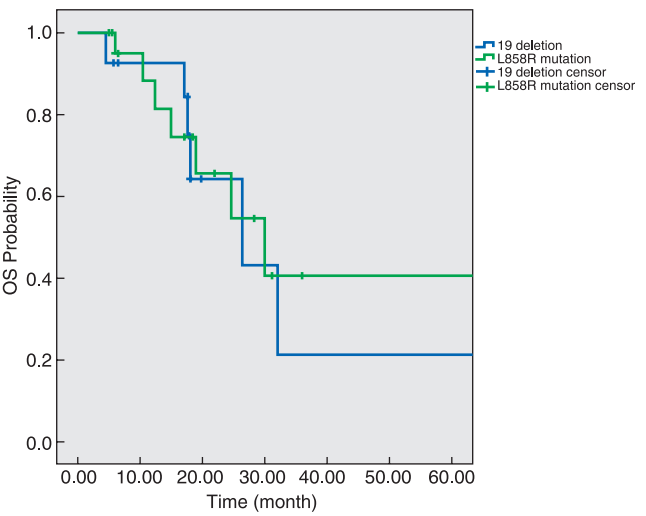

Fig. 4 OS comparison for patients *EGFR* 19 Del or L858R mutation

Asian population in the IPASS study [8].

Most of the patients (94.00%, 78/83) in our analysis with *EGFR* mutations received TKI treatment during the entire treatment period, and 63.86% (53/83) received first-line TKI for advanced NSCLC; this rate is clearly higher than that (48.68%) reported in the Guangdong Lung Cancer Institute and similar to that (66.30%) reported in the multicenter survey performed in the CTONG 1506 study [5–6]. These findings could be attributed to the fulfilment of clinical guidelines for managing EGFR mutation-positive NSCLC with the aid of medical insurance supporting TKI use in the Ordos area.

A previous meta-analysis showed that the *EGFR*-TKI therapy group of *EGFR* mutation-positive NSCLC patients had a significant improvement in progression-free survival (PFS) compared with the chemotherapy group, but the OS of the two groups did not differ significantly [9]. Most (94.00%) of the *EGFR* mutation-positive patients

in our study received TKI treatment, whereas only approximately one-third received both chemotherapy and TKI treatment during the entire process. In a previous study, *EGFR* mutation-positive patients who received first-line TKI and second-line chemotherapy achieved the highest OS of 30.39 months, compared with 20.67 months and 11.70 months for patients who received either TKI or chemotherapy alone, respectively, during the whole treatment period [10]. Our OS data supported these findings, with an average OS of 29.5 months for patients receiving both TKI and chemotherapy treatment, and were comparable to the results of phase III randomized, controlled clinical trials that reported OS times of 30.39 months and 27.7 months [10–11].

The treatment and survival data of our analysis represent the outcomes in real-world clinical practice because the patients' clinical characteristics in real-world practice differ from those in clinical studies, which have restrictive inclusion and exclusion criteria such as a required ECOG performance status (PS) of 0–2 and estimated life expectancy of at least 12 weeks and an absence of brain metastasis, history of cardiovascular disease, and uncontrolled pericardial or pleural effusion [10–11]. Our real-world population included patients with a range of conditions and only excluded those who could not tolerate or refused treatment.

Although the highest OS in our analysis (29.5 months) was not comparable with the 47.64 months obtained in patients with stage IV lung adenocarcinoma with *EGFR* mutations in the real-world study conducted by the Lung Cancer Mutation Consortium, which selected target treatments according to test results for 10 driver genes [12], it nevertheless represents an advancement in the TKI era. Moreover, the survival of female Asian stage IV lung adenocarcinoma patients in the Surveillance Epidemiology and End Results database was reported to increase from 8 months to 14 months from the pre-TKI era to the TKI era [13]. Additionally, patients treated with TKI in our database had notably longer OS times compared with that (13.9 months) of patients diagnosed with non-squamous NSCLC who received first-line pemetrexed maintenance treatment in the PARAMOUNT study [14]. This finding showed the importance of continuously improving *EGFR* testing and TKI treatment rates to prolong OS in *EGFR* mutation-positive patients (Fig. 1 and 2). Increased opportunities to administer TKI treatment may increase OS times for such patients.

As shown in Fig. 3, only around one-third of the patients in our database received both chemotherapy and TKI treatment, whereas 63.86% (53/83) received TKI treatment alone. This can be explained at least in part by the fact that first-line TKI therapy beyond progression is feasible but may delay salvage therapy for *EGFR* mutation-positive NSCLC, and is recommended as a basic strategy for cancer showing local progression or slow progression in the Chinese Society of Clinical Oncology guidelines [15–16]. Additionally, the TKI treatments gefitinib, erlotinib, and icotinib are provided by charitable organizations in mainland China. Therefore, patients with disease progression after first-line TKI treatment prefer to continuously apply for free TKI treatments rather than undergoing chemotherapy. Finally, the higher percentage of older patients and those with low PS status in real-world clinical practices compared with clinical trials contributes to the fact that most patients only receive TKI treatments.

Our OS of 26.5 months for patients who only received TKI treatment clearly exceeds the 20.67 months reported in the optimal study for similar patients [10]. This could be explained by the smaller sample sizes in our study. In several cases, the OS exceeded 60 months, which may explain why the Kaplan-Meier survival curves remain level after follow-up beyond 40 months in the TKI-only treatment group. In another study, encouraging PFS times were obtained for patients with T790M-positive advanced NSCLC who were pretreated with EGFR-TKI and received osimertinib, a third-generation TKI, after disease progression [17].

A previous pooled analysis of two multicenter, randomized clinical studies (Lux-lung 3 and Lux-lung 6) showed that *EGFR* 19 del and L858R mutations were considered as causative factors for two diseases that required different treatment strategies because of their distinct OS benefits with first-line TKI compared with first-line chemotherapy [18]. In this retrospective analysis, we compared the OS of 36 patients harboring *EGFR* 19 del or L858R mutations without brain metastasis at diagnosis who only received TKI treatment; however, the difference was not significant. Although our small sample size may have reduced the statistical power of the OS comparison, this is nevertheless in concordance with findings from Peking University Cancer Hospital [19]. In contrast, EGFR-TKIs provided a significant OS benefit to patients harboring 19 del compared with L858R mutations as reported in another study [20]. In our study, the OS of patients with L858R mutations was longer than that of patients with *EGFR* 19 del (26.5 months). One possible reason for this discrepancy is that more patients harboring L858R received third-generation TKI analogues after experiencing disease progression on first-line TKI treatment. To some degree, the different therapeutic effects of EGFR-tyrosine kinase inhibitors for 19 del and L858R mutations were more realistically reflected in this retrospective study because the patients in our analysis without brain metastasis at diagnosis who only received TKI had an OS level that was not affected by chemotherapy.

In conclusion, this retrospective study described

the results of a 5-year follow-up of stage IV lung adenocarcinoma *EGFR* mutation testing and treatment survival status in the Ordos area from a real-world viewpoint. Higher OS times were clearly attributed to the continuous improvements in *EGFR* testing and first-line TKI treatment rates. In the TKI era, the importance of chemotherapy in lengthening OS times should also be emphasized, because it did not only play an important role in whole process management but also showed a higher efficacy in managing TKI-resistant NSCLC when chemotherapy is given in combination with TKI [10, 21]. Differences in OS between patients harboring *EGFR* 19 del or L858R mutations should be analyzed further using a large data set.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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ORIGINAL ARTICLE

Clinicopathological characterization of gastroenteropancreatic neu-roendocrine neoplasms: a retrospective study of 48 cases

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Abstract

Objective Gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) constitute a rare and heterogeneous group of tumors with varied biology and still constitute a diagnostic and therapeutic challenge for physicians of all specialties. In the present study, we aimed to review and study the clinicopathological characteristics of GEP-NENs applying the World Health Organization (WHO) 2010 grading criterion.

Methods A total of 48 patients were enrolled in the study. The study included patients diagnosed with GEP-NENs who were treated and followed up at our Hospital from January 2013 to December 2017. Data regarding clinicopathological features of the patients were retrospectively evaluated. The expression of neuroendocrine markers was measured using the immunohistochemical *Ultra Sensitive™ S-P* method of staining in 48 cases of primary GEP-NENs; and serum levels of neuron-specific enolase, carbohydrate an-tigen 19-9, and carcinoembryonic antigen in 36 GEP-NEN patients were measured using the electrochemiluminescence method.

Results The median age at presentation was 59.3 (range 48–82) years, and 39 cases (81.3%) were seen between the 5th and 6th decades. There was a male predilection (male: female=3:1). In 79.2% cases (38/48), tumors were hormonally nonfunctional. The most common presentation was abdominal pain, and the most frequent primary site of the tumor was the rectum, followed by the stomach (n = 15, 31.3%), colon (n = 5, 10.4%), and so on. Of the 48 tumors, 16 (33.3%) were G1, 6 (12.5%) cases were G2, 16 (33.3%) were neuroendocrine carcinoma (NEC), and 10 (20.8%) were mixed adenoneuroendocrine carcinoma (MANEC). According to the AJCC/UICC classification, 45.8% (n = 22) were diagnosed at low stage (stage I or II) while 54.2% (n = 26) were diagnosed at high stage (stage III or IV) (the majority of NEC, G3, and MANEC). A male preponderance was noted for all tumors except for G2 neoplasms, which showed no gender predilection. Thirty-nine patients underwent endoscopic biopsy. The lesions in 18.8% (n = 9) of the patients were indentified only radiologically. After the surgical procedures, 36 had at least one follow-up visit with a median follow-up duration of 5 months; the mean follow-up period was 28 ± 16 months. The one-year and three-year survival rates were 72.2% (26/36) and 61.1% (22/36), respectively. This study did not find an effect of grade 3 (G3) of tumor on the short-term clinical outcome of these patients. In the survival analysis, NEN G3, higher stage (stage III or IV) according to the AJCC/UICC classification (P < 0.05), and metastases at diagnosis (P < 0.05) were associated with poorer prognosis.

Conclusion Most GEP-NENs are nonfunctional and nonspecific in presentation. The most frequent primary site of the tumor was the rectum and the commonest ages at diagnosis were the 5th and 6th decades. Endoscopic biopsy is the main diagnostic and histological grading method for GEP-NEN. In the survival analysis, NEN G3, a higher stage according to the AJCC/UICC classification, and metastases at diagnosis are associated with poorer prognosis.

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Key words: Gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs); Ki 67/MIB-1 index; mitotic rate; diagnosis; prognosis

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Neuroendocrine neoplasms (NENs) are tumors arising from the neuroendocrine cells which are distributed throughout the body. Gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) were originally identified as rare diseases occurring in the gastrointestinal tract and pancreas and displaying distinctive histopathological features from those of conventional gastroenteropancreat-ic epithelial cancers [1–2]. GEP-NENs refer to a group of heterogeneous cancers of neuroendocrine cell phenotype that mainly fall into one of two subtypes: gastroenteropancreatic neuroendocrine tumors (GEP-NETs) or gastroenteropancreatic neuroendocrine carcinomas (GEP-NECs), and are a highly heterogeneous and poorly understood group of rare but increasingly prevalent tumors with varied clinical presentation [3–4]. Most GEP-NENs, however, are nonfunctional and have non-specific presentations, which makes their early diagnosis challenging [1, 3]. They still constitute a diagnostic and therapeutic challenge for physicians of all specialties [1–2, 4–5].

Materials and methods

Diagnostic criteria for GEP-NENs

According to the World Health Organization (WHO) 2010 classification, GEP-NENs are classi-fied as NET Grade 1 (G1) and NET Grade 2 (G2) (well-differentiated endocrine tumors), and NEC Grade 3 (G3) (poorly differentiated endocrine carcinoma) [1]. The WHO 2010 classification takes into account the mitotic rate (usually expressed as mitoses per 10 high power microscopic fields or per 2 mm) and/or Ki-67 index (the percentage of neoplastic cells immunolabeled for the proliferation marker Ki-67) when grading GEP-NENs. Tumors with a Ki-67 index of < 2% or a mitotic rate of < 2/10 HPF are classified as G1, those with a Ki-67 index of 3–20% or a mitotic rate of 2–10/10 HPF are classified as G2, and those with a Ki-67 index of > 20% or a mitotic rate of > 20/10 HPF are classified as G3 [6–7] (Table 1).

Patients

This study included all cases of GEP-NEN involving the stomach, duodenum, jejunum, ileum, appendix, colon, rectum, and pancreas that were treated and followed up at our hospitals from January 2013 to December 2017. A total of 48 cases were enrolled in the study; among them, there were 39 patients from Rizhao People's Hospital, 5 from Rizhao Lanshan District People's Hospital, and 4 from Weihaiwei People's Hospital. The expression of neuroendocrine markers and Ki-67 was measured using the immunohistochemical *Ultra Sensitive™ S-P* method of staining in 48 cases of primary GEP-NENs; and the levels of neuron-specific enolase (NSE), carbohydrate antigen (CA) 19-9 and carcinoembryonic antigen (CEA) in 36 cases of gastrointestinal neu-roendocrine neoplasm patients were measured using the electrochemiluminescence method. All data regarding clinicopathological features and follow-up information were reviewed and evalu-ated. Of the 48 cases, 39 cases included endoscopic biopsies, and 48 cases had resection speci-mens. Based on WHO 2010 classification of GEP-NENs, all cases were graded as G1, G2 or G3. GEP-NENs mainly fall into one of two subtypes: well-differentiated GEP-NETs, or poorly dif-ferentiated GEP-NECs, and mixed adenoneuroendocrine carcinoma (MANECs). All the clinical and follow-up information were reviewed and evaluated, and their relationship with well-known clinicopathological factors such as tumor size, grade, lymph node status, and stage were investigated in GEP-NETs patients. The patients diagnosed with GEP-NETs had not been treated with hormone endocrine therapy, anti-neoplastic chemotherapy or radiotherapy during the pre-ceding six months. The follow-up details which were available until the end of the study period were collected. Permission was obtained from the local ethical committee to collect GEP-NET tissues and all patients signed informed consent forms prior to enrolment in the study.

Pathologic study

In this study, pathological diagnoses were made after histological staining of surgically resected or endoscopically biopsied tumor samples, and independently verified histologically by two pathologists, and pathological categorization was determined according to the current WHO clas-sification system diagnostic criteria (2010) [1]. The histopathological features and immunohisto-chemistry

Table 1 WHO 2010 classification of GEP-NETs

Grade	Two grade categories equivalent in WHO classification, 2010	Ki 67/MIB-1 index (%)	Mitotic rate (/10 HPF)
NET Grade 1	Well-differentiated endocrine tumors	< 2	< 2/10 HPF
NET Grade 2		3–20	2–20/10 HPF
NEC Grade 3 or MANC Grade 3	Poorly differentiated endocrine carcinoma	> 20	> 20/10 HPF

Note: NET: neuroendocrine tumor; NEC: neuroendocrine carcinoma; MANEC, mixed adenoneuroendocrine carcinoma; HPF, high power fields

details of all 48 cases were analyzed. The expression of neuroendocrine markers, such as CD56, chromogranin A (CgA), synaptophysin (Syn), NSE, cytokeratin (CK) 7, and Ki-67 were measured by the immunohistochemical method in 48 cases of primary GEP-NETs, 48 cases of dysplasia tissue closely adjacent to carcinomas, and 40 cases of normal colorectal mucosal specimens with complete clinical data from 2013 to 2017. All specimens were fixed in formalin and embedded in paraffin. Serial sections (4 μm) were deparaffinized in xylene and hydrated through a graded series of ethanol. The specimens were washed in phosphate-buffered saline within five minutes and examined under a binocular dissecting microscope. Immunoreactions were processed using the Ultra Sensitive™ S-P kit (Maixin-Bio, China) according to the manufacturer's instructions, and signals were visualized using the 3, 3'-diaminobenzidine substrate, which stains the target protein yellow. Negative controls were used. The primary antibody was replaced with phosphate-buffered saline, containing 0.1% bovine serum albumin of the same concentration as the primary antibody. The positive controls were tissues known to express the antigen being studied. CD56, CgA, Syn, NSE, CK7, and Ki-67 immunoreactivity expression was evaluated as the percentage of cancer cells that showed cytoplasmic staining reactivity. For Ki-67 expression, the percentage of cancer cells showing nuclear reactivity was recorded after inspection of all optical fields at 200× power and the mean value was used to score each case. Assessment of the staining was evaluated by two independent pathologists blinded to the clinical statuses of the patients.

Measurement of biomarkers in serum

The serum concentrations of NSE, CA 19-9 and CEA were measured using the electrochemilu-minescence immunoassay from Roche according to the manufacturer's instructions (Roche Diag-nostics, Germany). Three milliliters of blood was drawn from each patient and heparinized. The biomarker levels were detected in 36 cases of GEP-NET using the electrochemilu-minescence method in the clinical laboratory of Rizhao People's Hospital. The cut-off values of NSE, CA 19-9 and CEA in serum are 16.3 ng/mL, 27 U/mL and 3.40 ng/mL, respectively. For the biomarker levels, patients are divided into two groups (normal level or high-level peripheral blood). Serum levels of NSE, CA 19-9, and CEA above 30 ng/mL, 27 U/mL and 5 ng/mL, respec-tively, were considered as significantly elevated. In the case of multiple measurements, the high-est level was reported. The expression of Ki-67 proliferation index; the levels of NSE, CA 19-9 and CEA; and histological grade, regional lymph node metastasis, distant metastasis and recur-rence on record were also assessed in order to study the clinical and pathological characteristics associated with GEP-NETs.

Statistical analysis

Measurement data expressed as the mean and standard deviation (mean ± SD) between groups were compared using the *t*-test, while categorical data were compared using the chi-square (χ^2) test. *P* values less than 0.05 were considered statistically significant. All statistical analyses were performed using SPSS version 17.0 (SPSS Inc., USA).

Results

Clinical features

There were 36 male cases and 12 female; overall, there was male predilection (male: female = 3:1) in this study. Grade 1 tumors showed no male predilection (male: female = 9:7) as compared to the other grades (male: female = 27:5). The median age at presentation was 59.3 (range 48–82) years. Thirty-nine cases (81.3%) were seen between the 5th and 6th decades. The study patients had a delay of 2 (0–16) months from their first symptoms to their final diagnosis at the hospital. In 38 cases (79.2%), the tumors were hormonally nonfunctional. The most common presentation was abdominal pain, which was seen in 68.8% (33/48) of patients, followed by altered bowel habits (14/48, 29.2%), loss of weight and appetite (13/48, 27.0%), and abdominal mass (5, 10.4%). The most frequent primary site of the tumor was the rectum (n = 20, 41.7%), followed by the stomach (n = 15, 31.3%), colon (n = 5, 10.4%), pancreas (n = 4, 9.5%), small intestine (n = 3, 6.25%), and appendix (n = 1, 2.1%). These data were shown in Table 2.

Serum concentrations of biomarkers

The serum concentrations of CEA, NSE, and CA 19-9 are shown in Table 3. In the case of multi-ple measurements, the highest level was reported. The serum NSE and CEA levels were signifi-cantly higher in the poorly differentiated GEP-NEN groups than the well-differentiated groups (both $P < 0.05$), and the serum CA 19-9 levels were not significantly different between the groups (both $P > 0.05$). There were no significant differences in CEA, NSE and CA 19-9 levels between the GEP-NEN G1 and G2 groups; there were also no significant differences between the GEP-NEN NEC and MANEC groups (both $P > 0.05$). Compared with the group with Ki-67 index less than twenty percent, the serum levels of CEA, NSE, and CA 19-9 were significantly higher in the group with Ki-67 index more than twenty percent ($P < 0.05$).

Endoscopic and radiological findings

Among the 48 patients, 39 underwent endoscopy and so had available findings. In 39 (81.3%) patients, the

Table 2 Clinical characteristics of the GEP-NEN cases in the study group (n = 48)

Characteristic	n
Gender	
female	36
male	12
Age at diagnosis	59. 3 (48–82)
< 50 years	2
5th decade	19
6th decade	20
> 60 years	7
Hormonal activity	
Nonfunctioning NEN	38
Functioning NEN	10
Diagnosis method	
Endoscopy	39
CT	9
Primary tumor site	
stomach	15
small intestine	3
colon	5
rectum	20
appendix	1
pancreas	4
Grade (WHO classification, 2010)	
NEN G1	16
NEN G2	6
NEC G3	26
NEC	16
MANEC	10
AJCC/UICC classification	
Low stage (I or II)	22
High stage (III or IV)	26

Note: NEC, neuroendocrine cancer; NEN,neuroendocrine neo-plasm; AJCC/UICC, American Joint Committee on Cancer/Union for International Cancer Control; CT, computed tomograph

Table 3 Serum biomarker levels in the GEP-NEN cases in the study group (n = 36)

Grade	n	NSE ng/mL	A8 U/mL	CEA ng/mL
Well-differentiated	13			
NET G1	9	63. 7 ± 27. 2	37. 8 ± 21. 2	16. 9 ± 7. 9
NET G2	4	84. 3 ± 32. 9	39. 3 ± 23. 4	29. 8 ± 14. 2
Poorly differentiated	23			
NEC	15	98. 8 ± 48. 6	39. 7 ± 22. 9	42. 8 ± 19. 8
MANEC	8	122. 8 ± 75. 3	42. 3 ± 28. 7	53. 3 ± 22. 6

primary site was identified by endoscopic biopsy; in the remaining 9 (18.8%) patients, probable primary lesions were identified on radiological examination alone. Computed tomography (CT) scan showed a single mass 0.6–10.7 cm in maximum dimension; the largest lobulated mass 10.7 cm in maximum dimension was identified in the abdominal pancreas. CT scan showed that 2 patients had local mucosal destruction of the digestive tract wall which was interrupted, 2 patients had unevenly thickened lesions, 2 patients had annular thickened lesions, and in 3 patients the serous surface was clear with no tumor involvement. Upon enhancement, 2 patients had obvious enhanced lesions; enlarged lymph nodes could be seen in 4 patients (Fig. 1).

Pathological findings

Gross examination

Of the 48 study samples, 36 were resection samples available for gross examination and the re-maining 12 were endoscopic biopsy samples. Of the 36 cases, the cut surface of the tumor in all resection specimens had a single tumor nodule, ranging in size from 0.6 cm to 10.7 cm in maxi-mum dimension with a soft grey-white to yellow cut surface. Focal areas of hemorrhage were seen in 3 cases; grey-white zones with focal areas of necrosis were seen in 4 cases. There was no evidence of gross vascular invasion. The surrounding tissue was normal.

Histopathology

Histologically, the low grade tumors (G1 and G2) had classical patterns of arrangement including nests (n = 23), cords (n = 12), trabeculae (n = 18), festoons (n = 22), ribbons (n = 10), sheets (n = 8), gyriform (n = 6), acinar (n = 6), and pseudopapillary (n = 3) patterns. The cells were round to polygonal with moderate to abundant amounts of eosinophilic granular cytoplasm, and uniform to mildly pleomorphic nuclei with uniformly dispersed coarse chromatin and inconspicuous mitotic activity (mitotic rate: 0–10/10 HPF). The high-grade tumors (GEP-NECs and G3) showed sheet and nest patterns. The cells were medium to large sized, polygonal, with scanty to moderate amounts of eosinophilic cytoplasm, with mild to moderately pleomorphic nuclei, and with finely dispersed chromatin. There was increased mitotic and apoptotic activity (mitotic rate: 11–56/10 HPF) in NECs. Ten cases of MANEC had unequal adenoid structure, morphology consistent with small cell carcinoma with sheets and nests of polygonal cells displaying moderate nuclear pleomorphism and increased mitotic and apoptotic activity (mitotic rate: 16–56/10 HPF) (Fig. 2).

Immunohistochemistry

Immunostaining for neuroendocrine markers (CD56, CgA, Syn, and NSE), CK7 and Ki-67 were carried out in all 48 cases. CgA was positive in 32 (66.7%), Syn positive in 37 (77.1%) cases, NSE in 29 (60.4%) cases, and CD56 in 37 (77.1%) cases. CK7 immunostaining was performed in 12 cases with poorly differentiated neoplasms and showed positive staining in adenoid structure with G3 tumor of MANEC. A mean Ki-67 proliferation index of 10% (range 0–19%) in well-differentiated endocrine

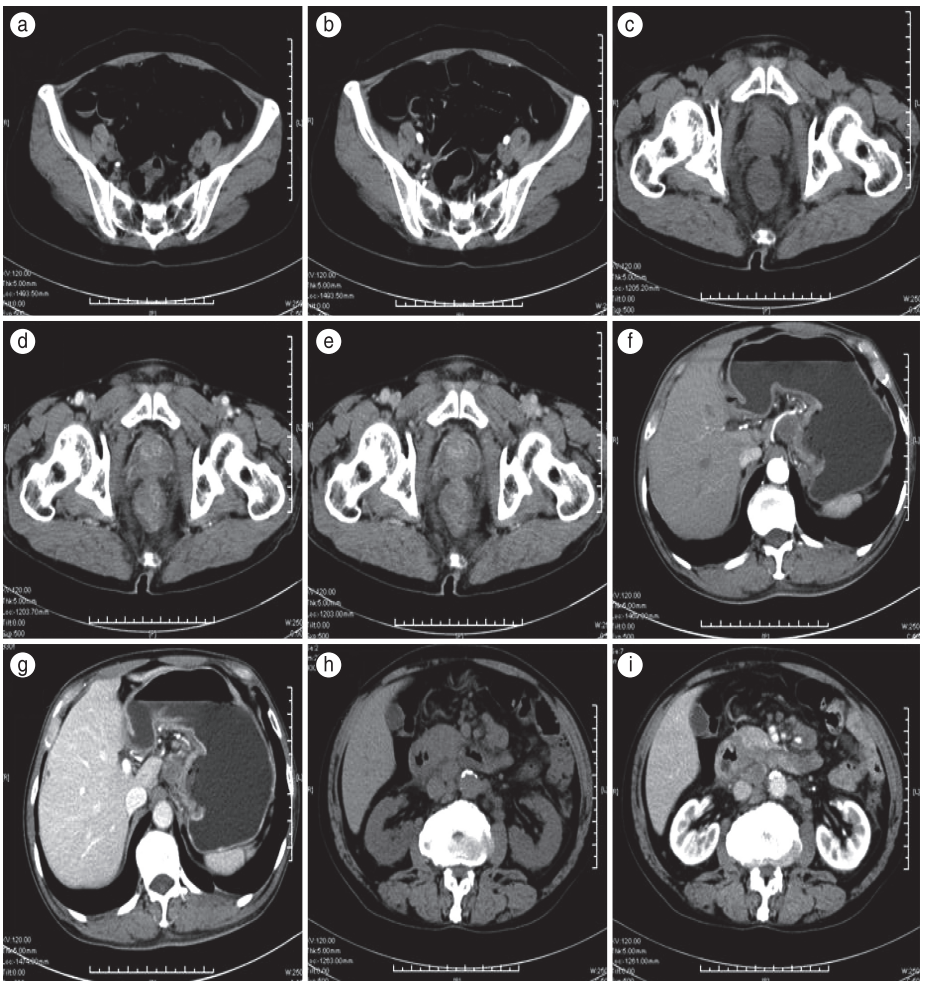

Fig. 1 NEN image findings. (a, b) sigmoid colon NET G1, the colon wall was locally thickened, obviously enhanced, and the serous surface was clear. (c–e) rectal NEC G3, the rectal wall was significantly annularly thickened and inhomogeneously enhanced with an indistinct serous surface. (f, g) stomach NEC G3, The gastric wall was thickened, the mucosa was interrupted, and enlarged lymph nodes were seen. (h, i) MANEC G3, the wall of the descending duodenal segment was thickened, with uneven thickness and obvious uneven enhancement.

tumors (WHO G1 and G2) and 25% (range 0–80%) in poorly differenti-ated endocrine carcinoma (WHO G3). Ten cases of MANEC had a mean Ki-67 proliferation index of 20% (range 10–70%) in the adenoid structure area and 50% (range 10–80%) in the endo-crine carcinoma area. The expression of Ki-67 in endocrine carcinoma and MANEC tissues was obviously higher than that in adjacent tissue and normal mucosal tissue (both $P < 0.05$). Ki-67 pro-liferation was significantly correlated with the medians of mitotic, and Ki-67 proliferation and the medians of mitotic were both significantly correlated with the grading (G3 vs G1, 2), stage and lymph node metastasis and distant metastasis (each $P < 0.05$) (Fig. 2).

Grade

Based on WHO 2010 grading of the 48 tumors, 16 (47.7%) were G1, 6 (12.5%) cases were G2, 16 (47.7%) were NECs, and 10 (20.8%) were MANECs, as WHO G3. According to the Ameri-can Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) classifica-tion, 45.8% ($n = 22$) were diagnosed at low stage (stage I or II), 54.2% ($n = 26$) were diagnosed at high stage (stage III or IV) (the majority of NEC G3 and MANEC). A male preponderance was noted in all tumors except for G2 neoplasms, which showed no gender predilection.

Follow-up

After the surgical procedures, 36 of the 48 patients had at least one follow-up visit with a median duration of follow-up of 5 months; the mean follow-up period was 28 ± 16 months. The one-year and three-year survival rates were determined to be 72.2% (26/36) and 61.1% (22/36), respective-ly. In the survival analysis, NEN G3, higher stage (stage III or IV) according to the AJCC/UICC

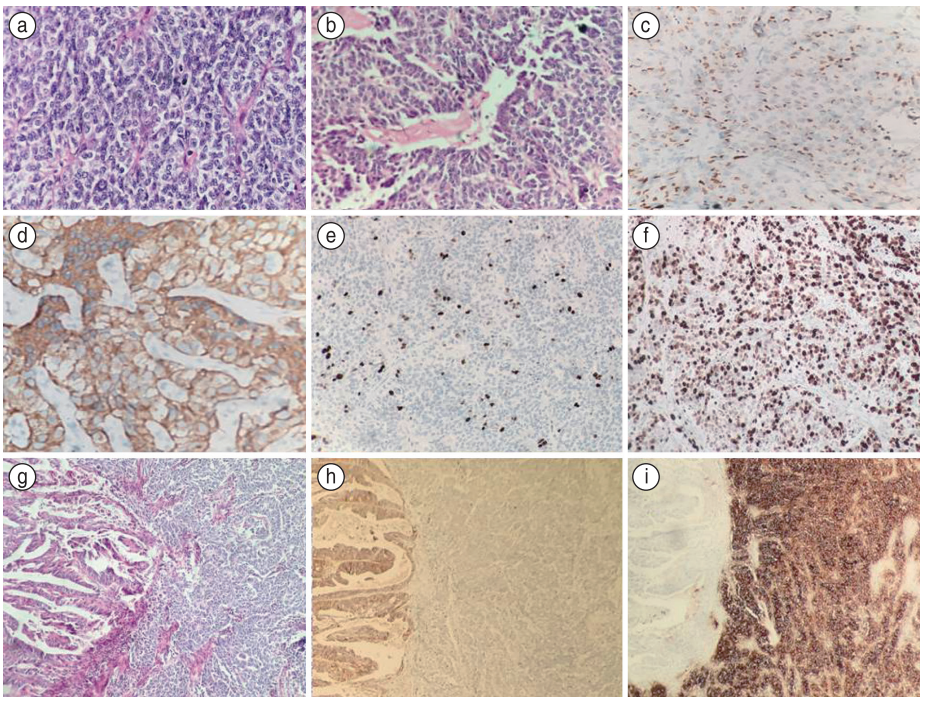

Fig. 2 NEN Histopathology and Immunohistochemistry. Histologically, the cells were round to polygonal with moderate to abundant amounts of eosinophilic granular cytoplasm, and uniform to mildly pleomorphic nuclei (a and b, HE stain). Immunostaining showed positive findings for neuroendocrine markers CgA (c) and Syn (d); Ki-67 proliferation index was less than 2% (e) and 70% (f) (Ultra SensitiveTM S-P stain); (g, i) MANEC G3, histological structure of mixed adenocarcinoma (left area) and neuroendocrine carcinoma (right area) (g, HE stain), CK7 positive in adenocarcinoma (h) and NSE positive in neuroendocrine carcinoma (i) (Ultra Sensitive™ S-P stain).

classification ($P < 0.05$), and metastases at diagnosis ($P < 0.05$) were associated with poorer prog-nosis. There was no significant correlation with sex, site, and age at diagnosis ($P > 0.05$).

Discussion

As mentioned, GEP-NENs are largely divided into GEP-NETs and GEP-NECs, according to the classification criteria defined by the WHO [1–2]. The annual global incidence of NEN has increased, with a fivefold increase over the past 30 years in the United States, possibly due to improvements in endoscopic cancer screening. This increase in the incidence of GEP-NENs has resulted in greater attention being paid to these diseases [1–2, 5]. In our study, there was a male predilection (male: female = 3:1). NET G 1 showed no male predilection (male: female = 9:7) as compared to the other grades (male: female = 5.4:1). The median age at presentation was 59.3 (range 48–82) years, and 81.3% were seen between the 5th and 6th decades. In our study, 79.2% of tumors were hormonally nonfunctional. However, the serum NSE and CEA levels were significantly higher in the poorly differentiated GEP-NEN groups than the well-differentiated groups, and the serum CA 19-9 levels were not significantly different between the groups. There were no significant differences in CEA, NSE and CA 19-9 levels between the GEP-NEN G1 and G2 groups, and there were also no significant differences between the GEP-NEN NEC and MANEC groups. In this study, the most common presentation was abdominal pain, which was seen in 68.8% of patients. In our study, the most frequent primary site of the tumor was the rectum, which is consistent with other reports [6], followed by the stomach, colon, pancreas, small intestine, and the appendix had the lowest incidence in our study group. The most common primary tumor site in most reports from Europe and the United States was the small intestine [1–2, 7–8]. However, in Asian epidemiological surveys, rectal NENs were more frequent [6].

A GEP-NEN diagnosis is based on the loss of epithelial tubular gland structures [9], the diffuse expression of neuroendocrine markers (particularly of CgA, Syn, and CD56) and the proliferative cell rate, as represented by the Ki-67 index and the mitotic count [1–2, 10–12]. In this study, histolog-ical structures such as festoons, nests, trabeculae, cords, ribbons, sheets, gyriform, acinar, and pseudopapillary were all seen. Pseudopapillary patterns were seen in 3 cases of pancreatic tumor. The cells were round to polygonal with moderate to abundant amounts of eosinophilic granular cytoplasm, and uniform to mildly pleomorphic nuclei with uniformly dispersed coarse

chromatin and inconspicuous mitotic activity. Our study group showed the high-grade tumors (GEP-NEC G3) with mainly sheets and nests patterns and the cells were medium to large sized, polygonal, with scanty to moderate amounts of eosinophilic cytoplasm, mild to moderately pleomorphic nu-clei, with finely dispersed chromatin. There were increased mitotic and apoptotic activities in NECs compared to well-differentiated endocrine tumors. Neuroendocrine markers are immuno-reactive markers for diagnosis and indicate the neuroendocrine differentiation of tissue. CgA, Syn, NSE and CD56 as a biomarker panel for GEP-NENs can improve the sensitivity of diagnosis of GEP-NENs complementarily. CgA, Syn and CD56 are used as neuroendocrine markers for GEP-NENs. CgA is a neuroendocrine secretory protein, Syn is a synaptic vesicle glycoprotein present in neuroendocrine cells and CD56 is a neural cell adhesion molecule. In this study, CgA was positive in 66.7%, Syn positive in 77.1% cases, NSE in 60.4% cases and CD56 in 77.1% cases. In our study group, GEP-NENs diffusely expressed at least one neuroendocrine marker. In our study, MANEC had unequal adenoid structure and morphology consistent with small cell carcinoma with sheets and nests of polygonal cells displaying moderate nuclear pleomorphism and increased mitotic and apoptotic activity.

Ki-67 and mitotic activity are two markers used in the subclassification of GEP-NENs [1–2]. The GEP-NENs have been classified by the WHO (2010) in three grades (G1 to G3) based on mitotic activity and Ki-67/MIB-1 proliferation index [1]. These are G1: mitotic count < 2/10 HPF and/or Ki-67 proliferation index ≤ 2%. NEN G2 cells have a Ki-67 index of 3–20% and/or a mitotic count of 2–20 per 10 HPF. NET G1 and G2 cells are well-differentiated, the cells are round to polygonal with moderate to abundant amounts of eosinophilic granular cytoplasm, and uniform to mildly pleomorphic nuclei with uniformly dispersed coarse chromatin. However, GEP-NEC G3 cells are poorly differentiated and defined as NEC with mitotic count > 20/10 HPF and/or Ki-67 proliferation index > 20%. If the mitotic count or Ki-67 proliferation index points to different grades, a higher grade has to be given [9–12]. Some studies have shown discordance between mitot-ic count and Ki-67 index in some cases [8–10]. They have shown that the grade discordant tumors with a mitotic count of G1 and Ki-67 index of G2 behave worse than grade concordant tumors [9–10]. In our study, 33.3% of cases were G1, 12.5% were G2, 33.3% were NEC, and 20.8% were MANECs. Poorly differentiated tumors NEC and MANEC tend to have a higher Ki-67 index than do NET G1 and G2 tumor cells. Compared with the group with Ki-67 index less than twenty percent, the serum levels of CEA, NSE, and CA 19-9 were significantly higher in the group with Ki-67 index more than twenty percent in this study. In this study, the one-year and three-year survival rates were determined to be 72.2% and 61.1%, respectively. In the survival analysis, NEN G3, higher stage (stage III or IV) according to the AJCC/UICC classification ($P < 0.05$), and metastases at diagnosis ($P < 0.05$) were associated with poorer prognosis. There was no significant correlation with sex, site, and age at diagnosis ($P > 0.05$).

As a heterogeneous disorder, GEP-NETs can be located in various anatomic sites in the abdomen, resulting in a wide range of clinical pictures and requiring the further inclusion of relevant clini-cians. The management of GEP-NETs requires the accumulation of knowledge and experience to establish a standardized approach.

GEP-NENs constitute a rare and heterogeneous group of tumors with varied biology and still constitute a diagnostic and therapeutic challenge for physicians of all specialties. These findings demonstrate that most GEP-NENs tumors are nonfunctional and present with nonspecific symp-toms. The most frequent primary site of the tumor was the rectum, and the age at diagnosis was 5th and 6th decades. Endoscopic biopsy is the main diagnostic and histological grading method for GEP-NEN. In the survival analysis, NEN G3, higher stage (stage III or IV) according to the AJCC/UICC classification, and metastases at diagnosis were associated with poorer prognosis.

Conflict of interest

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

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ORIGINAL ARTICLE

N-myc downstream-regulated gene 2 promotes proliferation of HO-8910 ovarian cancer cells

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Abstract

Objective To investigate N-myc downstream-regulated gene 2 (NDRG2) expression in ovarian cancer cells and its potential usefulness as a diagnostic marker and/or target for therapeutic intervention.

Methods Human *NDRG2L/S* gene was obtained by revers-transcription polymerase chain reaction (RT-PCR). Sequence analysis confirmed the identity of *NDRG2L/S* gene, which was then inserted into a eukaryotic vector pLNCX2, which was in turn transfected into *NDRG2* gene-negative HO-8910 cells. Flow cytometry (FCM) and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay were conducted to determine the proliferation rate of HO-8910 cells. Cisplatin resistance of HO-8910 cells transfected with pLNCX2-NDRG2L/S was evaluated by FCM. Tumors were generated in female nude mice by subcutaneous injection of HO-8910 cells.

Results *NDRG2* gene was isolated and its expression vector was successfully constructed. *NDRG2* expression positively correlated with the proliferation of HO-8910 cells. *NDRG2L/S* promoted tumorigenicity in HO-8910 cells.

Conclusion The present study identified a novel function of *NDRG2L/S* gene and demonstrated its involvement in the promotion of ovarian cancer cell proliferation and enhancement of cisplatin resistance in HO-8910 cells. Future studies are warranted to determine the relationship between *NDRG2* upregulation and ovarian cancer progression.

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Key words: N-myc downstream-regulated gene 2 (NDRG2); ovarian cancer; HO-8910 cell; MTT; cisplatin

Ovarian cancer is the fifth most common cancer in females and the leading cause of mortality related to gynecological malignancies [1–2]. It is the second most common gynecological cancer following cancer of corpus uteri, with 21,980 and 67,000 new cases reported in the United States and Europe in 2014 [3] and 2008 [4], respectively. The global incidence rate of ovarian cancer involves 225,500 new cases and 140,200 deaths every year, including 14,030 deaths in the United States alone [5]. As ovarian carcinoma presents nonspecific symptoms and is often asymptomatic until later stages, majority of patients are not diagnosed until they reach advanced stages of the disease [6–7]. Ovarian cancer is a lethal gynecological malignancy, with more than 70% of women presenting advanced stage disease [8]. Standard of care for ovarian cancer is surgical debulking, followed by combination treatment with platin-based drugs such as carboplatin and paclitaxel [9]. Cisplatin was first approved by the Food and Drug Administration (FDA) for the treatment of ovarian cancer in 1978 [10]. Some evidence exists to support the success of neoadjuvant chemotherapy in women who present with advanced, unresectable primary ovarian cancer, followed by interval debulking; however, some results also suggest that this approach imparts little or no benefit [11]. Most women initially respond to these chemotherapeutic drugs, but the majority would relapse within 2 years, ultimately developing broad chemoresistance [12]. Despite new treatments, no significant changes in long-term outcomes have been reported in the past 30 years, and more than 60% of advanced stage patients develop recurrent disease [13].

NDRG2, a member of the *N-myc* downstream-regulated gene family, belongs to the alpha/beta hydrolase superfamily. It was first cloned from a normal human brain cDNA library by subtractive hybridization (GenBank Accession No. AF159092) and is regarded as a tumor suppressor gene transcriptionally repressed by c-Myc [14–16]. The human *NDRG2* gene, located at chromosome 14q11.2, comprises 16 exons and 15 introns and encodes

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for a 41-kDa protein. Two isoforms of *NDRG2* have been previously described, one of which contains a 42-bp insertion in the mRNA owing to alternative splicing that results in a protein carrying additional 14 amino acid residues[17]. In this study, these isoforms were isolated and named as NDRG2L and NDRG2S. The biological significance of these isoforms is currently unknown. It has been proposed that *NDRG2* is a candidate tumor suppressor gene and its expression is generally low or undetected in various tumors and tumor cell lines. To date, no report has described the relationship between *NDRG2* expression and ovarian cancer.

The objective of this study was to investigate *NDRG2* function in the ovarian cancer cell line HO-8910 and explore the effects of *NDRG2* upregulation on cisplatin resistance in HO-8910 cells. In this study, the human *NDRG2L/S* gene was obtained by reverse-transcription polymerase chain reaction (RT-PCR) and subjected to sequence analysis. Furthermore, a retroviral vector NDRG2 expression system was used to verify the effects of pLNCX2-NDRG2L/S on the proliferation of HO-8910 cells and growth of tumor in a nude mouse model.

Materials and methods

Cell culture and gene transfection

HO-8910 cells were cultured in Dulbecco's modified Eagle's medium (HyClone, USA) supplemented with 10% fetal bovine serum (HyClone), 100 U/mL penicillin, and 1 × penicillin-streptomycin (100 U/mL and 100 μg/mL, respectively) (Invitrogen, USA). Plasmids were introduced into cells using a pLNCX2 retrovirus vector (BD, USA) system, as per the manufacturer's protocol. The transfected cells were selected by G418 and continuously cultured until harvest and subsequent analysis.

RT-PCR and real-time quantitative RT-PCR (qRT-PCR)

Regular RT-PCR and qRT-PCR were performed as previously described on an ABI PRISM 7300 detection system (ABI, USA) using the primers listed in Fig. 1. The RT-PCR reactions were repeated at least thrice.

Western blotting

For western blotting, cells were lysed using 20 mM Tris–HCl (pH 8.0), 5% glycerol, 138 mM sodium chloride (NaCl), 2.7 mM potassium chloride (KCl), 1% NP-40, 20 mM sodium fluoride (NaF), 5 mM ethylenediaminetetraacetic acid (EDTA), 1 mM sodium orthovanadate, 5 mg/mL leupeptin, 1 mg/mL pepstatin, and 1 mM dithiothreitol. The extracted proteins were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) before transferring onto a polyvinylidene fluoride (PVDF) membrane, followed by incubation with anti-NDRG2 (ab57429, Abcam, USA) and anti-β-actin antibodies (Santa Cruz Biotechnology, USA).

Xenografting of ovarian cancer cells and tumor development in mice

Tumors were generated in nude mice (Vital River, China) by a subcutaneous injection of HO-8910 cells [5×10^5 cells in 100 μL of phosphate-buffered saline (PBS)] into the right dorsum of each mouse (10 mice in each group and a total of three groups). Tumor measurements were converted to tumor volume (V) using the formula ($L \times W^2 \times 0.52$), where L and W were the length and the width, respectively. Tumor growth was measured once every 2 days using a Vernier caliper. The mice were sacrificed on day 38, and the number and size of each tumor were macroscopically quantified. Macroscopic tumor images were acquired with a Canon camera and processed with Adobe Photoshop CS Version 8.0. All procedures were performed according to animal welfare and other related ethical regulations approved by the Institutional Animal Care Committee of Medical College at Xiamen University.

Data analysis and statistics

Data were presented as the mean ± standard deviation, as indicated for each figure. Statistical comparisons between groups were performed using the Student's *t*-test. A value of $P < 0.05$ was considered to indicate statistically significant differences.

Results

Modulation of NDRG2 expression by pLNCX2-NDRG2 in HO-8910 cells

To generate *NDRG2* mRNA, we cloned the *NDRG2* gene by RT-PCR (Fig. 1a). The pLNCX2-NDRG2 eukaryotic expression vector was obtained and confirmed by DNA sequencing (Fig. 1b). To determine the role of *NDRG2*, we used the HO-8910 cell line as the experimental model, as HO-8910 cells exhibit low endogenous *NDRG2* levels. The cells were infected with pLNCX2-NDRG2, and western blotting was used to evaluate NDRG2 upregulation. In comparison with the pLNCX2 group (negative control), cells treated with pLNCX2-NDRG2 showed upregulated expression of NDRG2 (Fig. 1c).

Proliferative effects of *NDRG2* overexpression on HO-8910 cells

To investigate the relationship between HO-8910 cells and *NDRG2* expression, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and colony formation assays were performed. The MTT

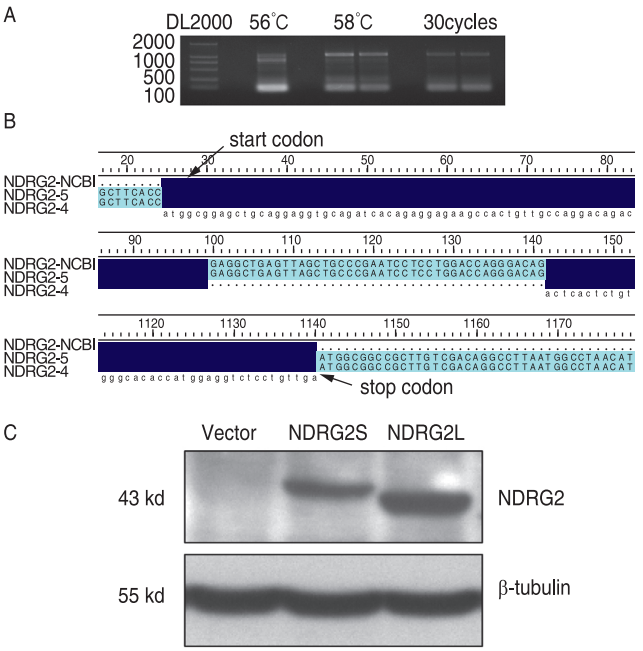

Fig. 1 Overexpression of NDRG2 in HO-8910 cells. (A) NDRG2 gene was cloned by RT-PCR; (B) The sequence of pLNCX2-NDRG2 eukaryotic expression vector was confirmed by DNA sequencing; (C) Western blot analysis of NDRG2 in HO-8910 cells infected with pLNCX2-NDRG2. Equal amounts of proteins were subjected to western blot analysis.

assay was designed with an OD gradient and infection time gradient (days 2, 4, and 6). We infected the cells with pLNCX2, pLNCX2-NDRG2L, or pLNCX2-NDRG2S; approximately 6 days later, the proliferation rate of the groups treated with pLNCX2-NDRG2L and pLNCX2-NDRG2S was significantly different from that of the control (Fig. 2a). Next, we assayed the contribution of pLNCX2-NDRG2L and pLNCX2-NDRG2S to colony formation in HO-8910 cells. Cells from each group were incubated for 2 weeks, followed by cell number enumeration. As shown in Fig. 2b, the colony formation ratio was significantly increased to 23.6% in cells treated with pLNCX2-NDRG2S as compared with that in the control group. In contrast, no obvious difference in the colony formation ratio was observed in the pLNCX2 group. These data revealed that NDRG2 overexpression promotes the proliferation of HO-8910 cells. Third, detection of the cell cycle changes by flow cycometry (FCM).

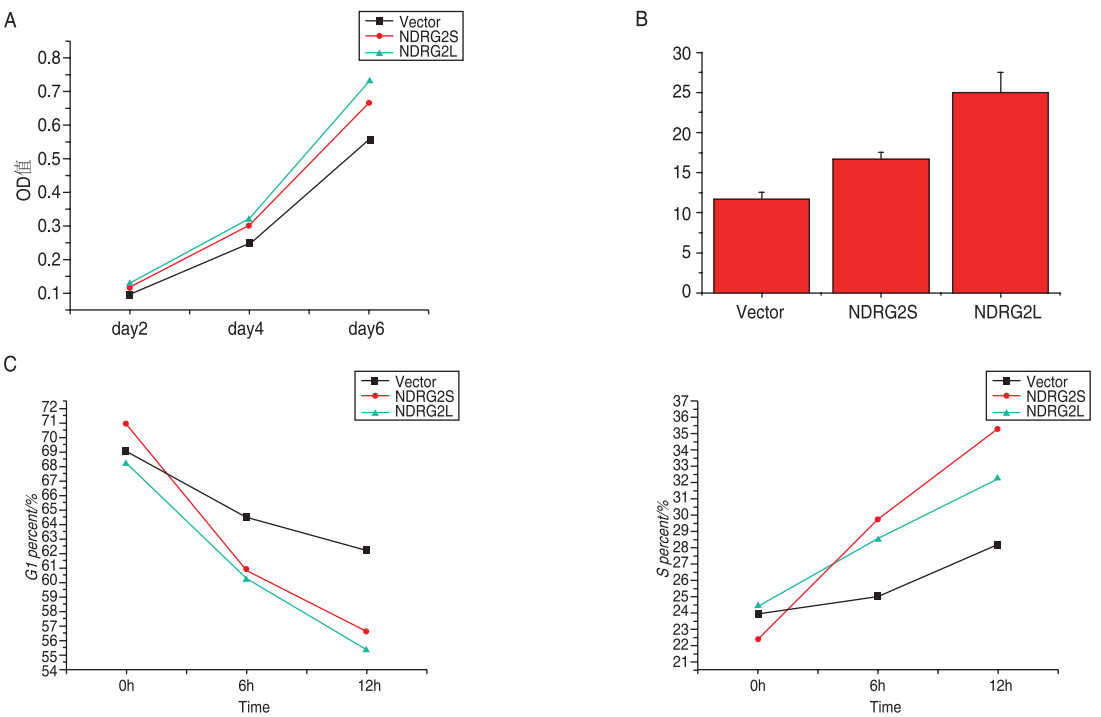

Fig. 2 NDRG2 overexpression promotes HO-8910 cell growth. (A) Time-dependent promotion of HO-8910 cell proliferation following pLNCX2-NDRG2-4 and pLNCX2-NDRG2-5 infection. After pLNCX2-NDRG2-4 and pLNCX2-NDRG2-5 infection, the cells were incubated for different time periods (2, 4, and 6 days). Cell proliferation was quantified using the MTT assay; (B) The effect of pLNCX2-NDRG2 on the colony formation ability of HO-8910 cells was examined. After pLNCX2-NDRG2-4 and pLNCX2-NDRG2-5 infection, the cells were incubated for approximately 10 days until colony formation was observed. Only the clearly visible colonies (diameter > 50 μm) were counted; (C) Detection of cell cycle changes by FCM.

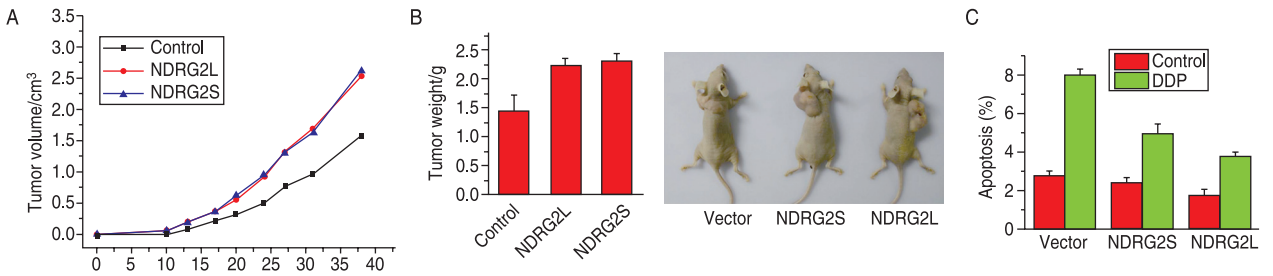

Fig. 3 Effects of pLNCX2-NDRG2 on the growth of HO-8910 cells xenografted into mice. (A) Tumor growth curve. Tumor growth was assessed every 3 days until day 38 by measuring two perpendicular diameters and calculating the volume in cubic centimeter. Statistical analysis was performed with values obtained on day 38 using one-way analysis of variance (ANOVA) and Student's *t*-test. ** indicates $P < 0.01$ as compared to the control; (B) Representative images of xenografted tumors; (C) *NDRG2* promotes resistance of HO-8910 cell lines to cisplatin (DDP)-induced apoptosis.

Promotion of tumor growth in a nude mouse model by intratumoral pLNCX2-NDRG2 injection

To investigate the effects of NDRG2 expression on the tumor growth *in vivo*, tumors were generated in nude mice by subcutaneous injection of HO-8910 cells (5×10^5 cells in 100 μL of PBS) that had been infected with pLNCX2, pLNCX2-NDRG2L, or pLNCX2-NDRG2S into the right dorsum of each mouse. As shown in Figure 3a and 3b, the tumors from pLNCX2-NDRG2L and pLNCX2-NDRG2S groups showed sustained and significant growth (mean tumor volume on day 38 of 2.80 and 1.45 cm^3, respectively). NDRG2 promoted the resistance of HO-8910 cells to cisplatin (DDP)-induced apoptosis (Fig. 3c).

Discussion

The expression of *NDRG2* is ubiquitous and particularly high in normal human tissues, while the bone marrow, testis, peripheral blood, and placenta exhibit relatively low *NDRG2* expression. *NDRG2* expression is almost undetectable in human pancreatic cancer, hepatocellular carcinoma [18], thyroid cancer, colorectal cancer [19], gastric cancer [20], and some human cancer cell lines such as those of breast, stomach, and colon [21]. The distinct expression patterns between normal and neoplastic tissues and cell lines suggest that *NDRG2* is a differentiation-related gene and may play a vital role in homeostasis. *NDRG2* has been identified as a prognostic marker in gastric cancer because of its significantly decreased expression, which, in turn, has been strongly associated with poor prognosis and low survival rates [22]. However, no report has described the relationship between NDRG2 gene and ovarian cancer.

Previous studies have shown that *NDRG2* overexpression reduces glioblastoma proliferation *in vitro* [23], while *NDRG2* silencing was found to enhance the proliferation of colon cancer and gastric cancer cells *in vitro* [24]. Furthermore, *NDRG2* overexpression suppresses human liver cancer invasion and migration *in vitro* and reduces metastasis *in vivo* [25]. These findings suggest that *NDRG2* may be an important malignancy factor. In our research, the MTT assay and FCM results showed that the upregulation in *NDRG2* expression increased the proliferation rate of HO-8910 cells. In addition, *NDRG2* facilitated the transition of HO-8910 cells from G0/G1 phase to S phase. *NDRG2* promoted the resistance of HO-8910 cells to cisplatin (DDP)-induced apoptosis. This finding is contradictory to the results of a previous study on the role of *NDRG2* in tumor metastases. The mechanism underlying this observation remains unknown.

In summary, the present study demonstrates for the first time that *NDRG2* overexpression induces proliferation in ovarian cancer HO-8910 cells *in vitro* and *in vivo*. Therefore, *NDRG2* gene may be a promising target for the development of novel therapeutics and may potentially play an important role in the prevention and treatment of ovarian cancer.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

The efficacy of capecitabine and temozolomide against neuroendocrine carcinomas

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Abstract

Objective Neuroendocrine carcinomas (NECs) are resistant to currently available chemotherapy agents, and its therapeutic options are limited. Preclinical data have suggested synergy between capecitabine and temozolomide (CAPTEM). Therefore, we evaluated the efficacy and safety of CAPTEM in patients with metastatic NECs who have failed prior therapies.

Methods A retrospective review was conducted on seven patients with metastatic NECs for whom platinum-based chemotherapies and hepatic chemoembolization failed. Patients received capecitabine (1000 mg twice daily on days 1-14) and temozolomide (150–200 mg/m^2 once daily on days 10–14) every 28 days. Tumor assessments were performed every two cycles.

Results Among the seven patients treated, two achieved partial remission and four achieved stable disease. The total response rate was 29%, and the clinical benefit was 86%. Median progression-free survival was 10 (range: 8–14) months. The most common toxicities were grade 1 and 2 neutropenia, grade 1 fatigue, and grade 1 and 2 hand-foot syndrome. No grade 4 toxicities or treatment-related deaths were observed.

Conclusion Our study showed that the CAPTEM regimen is an effective and well-tolerated salvage option for NECs. Further prospective studies are warranted to evaluate optimal combinations of the CAPTEM regimen for NECs.

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Key words: temozolomide, capecitabine, neuroendocrine carcinomas

Neuroendocrine tumors (NETs) are characterized by their ability to secrete peptides, resulting in distinctive hormonal syndromes. They represent a heterogeneous group of tumors with varying biological and clinical behaviors based on their functionality and differentiation. NETs account for 1–2% of all malignancies, and recent epidemiological studies have revealed an increasing incidence of this type of cancer [1].

The World Health Organization classified NETs based on their differentiation and Ki-67 rate in order to assess their biological behavior and potential for a malignant phenotype. Neuroendocrine carcinomas (NECs) are classified into fast-growing, poorly differentiated tumors, with Ki-67 rate of > 20%. NECs are highly heterogeneous, including small cell type, large cell type, and mixed type, and are a part of well-differentiated NETs. Different types of NECs have varied sensitivity to drugs and prognosis. NECs with a Ki-67 rate of ≥ 55% are more responsive to platinum-based chemotherapies, and those with a Ki-67 rate between 20% and 55% are less responsive to platinum-based chemotherapies [2].

Temozolomide is an oral alkylating agent, with a mechanism of action similar to dacarbazine. The therapeutic benefit of temozolomide depends on its ability to methylate DNA, which most often occurs at the N-7 or O-6 positions of guanine residues. This methylation damages the DNA and triggers the death of tumor cells [3]. *In vitro* studies have suggested a synergistic activity of CAPTEM, an oral form of 5-FU [4]. The mechanism of synergism is uncertain. However, the data suggest that the synergy is dependent on the sequence of the two drugs. Temozolomide should be administered after the exposure of tumor cells to capecitabine. One possible explanation for this synergy is depletion of the DNA repair enzyme O^6-methylguanine DNA methyltransferase (MGMT) by capecitabine, thereby reinforcing the effect

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of temozolomide [5].

The efficacy of second-line treatment for NECs with capecitabine and temozolomide (CAPTEM) has rarely been explored. In this study, we present a retrospective analysis on its treatment efficacy and safety in seven patients with metastatic NECs who received CAPTEM as second-line treatment at the Department of Oncology, Inner Mongolia People's Hospital.

Materials and methods

Data of seven patients diagnosed with metastatic NECs were retrospectively reviewed between January 2009 and January 2014. Patients received capecitabine (Xeloda, Roche, 1000 mg twice daily on days 1–14) and temozolomide (Diqing, Tasly Diyi, 150 mg/m^2 once daily, and increased to 200 mg/m^2 in cycle 2 if well tolerated, on days 10–14) every 28 days. Clinical and pathologic characteristics are listed in Table 1.

Imaging was performed every two cycles, and serum tumor markers were measured every cycle. Response to treatment was assessed using Response Evaluation Criteria in Solid Tumors (RECIST) parameters [6]. Toxicity was graded using the National Cancer Institute Common Terminology Criteria for Adverse Events [7]. All patients were followed until progression or death before these data were analyzed.

Results

Based on the RECIST parameters, two patients achieved partial response and four achieved stable disease. The total response rate was 29%, and the clinical benefit (responders and stable disease) was 86%. Median progression-free survival was 10 (range: 8–14) months. The combination regimen was generally well tolerated. Grade 3 toxicities included grade 3 hand-foot syndrome and thrombocytopenia in one patient. The most common toxicities were grade 1 and 2 neutropenia, grade 1 fatigue, and grade 1 and 2 hand-foot syndrome. No patient discontinued treatment because of toxicities, and no grade 4 or treatment-related deaths were observed. One patient required dose reductions because of grade 3 hand-foot syndrome (Table 2).

Discussion

In general, patients with metastatic NECs have a poor prognosis and short-term survival. The standard option for advanced disease is chemotherapy. However, few treatment strategies are effective for patients who experience treatment failure.

This study aimed to evaluate the efficacy and tolerability of CAPTEM regimen as second-line treatment after a

Table 1 Characteristics of the seven patients enrolled

Characteristics	*n*
Age, median (range, years)	47 (26–68)
Male/female ratio	2:5
ECOG performance status	
0	1
1	3
2	3
KI-67 index (20%–55%)	7
Primary tumor	
Pancreas	4
Gastric	1
Colon	1
Rectum	1
Site of metastases	
Liver	3
Lymph nodes	4
Lung	1
No. of metastatic sites	
1	6
2	1
Elevated tumor markers	
(Chromogranin A, 5-HIAA)	4
Resection of primary tumor	4
Previous TAE/TACE	2

Note: TAE: transarterial embolization, TACE: transarterial chemoembolization

Table 2 Adverse events

Adverse events	Grade 1	Grade 2	Grade 3	Grade 4
	No.	No.	No.	No.
Hematologic				
Anemia	1	1		
Neutropenia	5	2		
Thrombocytopenia	2	1	1	
Nonhematologic				
Nausea	2	1		
Vomiting	1	1		
Anorexia	2	1		
Diarrhea	1			
Fatigue	6			
Elevated AST	1	1		
Elevated ALT	1	1		
Hand-foot syndrome	3	2	1	

platinum-based chemotherapy in patients with NECs. We have observed a response rate of 29% and a clinical benefit rate of 86% among patients with metastatic NECs treated with CAPTEM regimen. The median progression-free survival was 10 months. No grade 4 toxicities were associated with this regimen. Grade 3 events were also limited. The dosage of our CAPTEM regimen was well tolerated with a good safety profile. The high clinical benefit rate and low toxicity rate in our study appear to validate this treatment strategy.

Among the four patients with pancreatic neuroendocrine carcinomas (PECAs), one achieved partial remission and three obtained a stable disease status. The synergism of CAPTEM is not fully understood. Preliminary evidence revealed that PECAs express low levels of MGMT [8], which perhaps explains the high level of chemosensitivity to temozolomide. In the future, more experiments should be designed to investigate whether MGMT expression in metastatic NECs correlates with response to CAPTEM.

The nuclear antigen Ki-67 may be a prognostic indicator and a surrogate marker [9]. Previous analysis showed a significantly shorter median survival in patients with a Ki-67 rate of ≥ 50%. The study on temozolomide-based chemotherapy against NECs also found more responders among patients with a Ki-67 rate of < 60% than among those with a higher Ki-67 rate [10]. This suggests that there are biological differences in the tumor between those with high and low Ki-67 rates.

Although the number of cases in our study is small, it triggers interest for future studies. In order to establish a standard regimen for NECs, a randomized study comparing CAPTEM and platinum-based treatments should be considered. In addition, to optimize the result of the investigation, patients should be selected based on the appropriate Ki-67 rate (< 55%).

Conflicts of interest

The authors declare to have no conflicts of interest.

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GUIDELINE OBSERVATION

Updates of the NCCN guidelines for head and neck cancers

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Updates in version 2.2018 of the NCCN guidelines for head and neck cancers from version 1.2018

ST-15

Staging table added.

Table 7
American Joint Committee on Cancer (AJCC)
TNM Staging System for Cervical Lymph Nodes and Unknown Primary Tumors of the Head and Neck (8th ed., 2017)
(Squamous cell carcinoma and salivary gland carcinoma of all head and neck sites except HPV-related oropharynx cancer, nasopharynx ca melanoma, thyroid carcinoma, and sarcoma. Staging of the patient who presents with an occult primary tumor and EBV-unrelated and HPV metastatic cervical lymphadenopathy is also included.)

Regional Lymph Nodes (N)		
Clinical N (cN): For patients who are treated with primary nonsurgical treatment without a cervical lymph node dissection.		
NX		Regional lymph nodes cannot be assessed
N0		No regional lymph node metastasis
N1		Metastasis in a single ipsilateral lymph node, 3 cm or smaller in greatest dimension and ENE(-)
N2		Metastasis in a single ipsilateral lymph node, larger than 3 cm but not larger than 6 cm in greatest dimension and ENE(-); or metastases in multiple ipsilateral lymph node(s), none larger than 6 cm in greatest dimension and ENE(-); or in bilateral or contralateral lymph nodes, none larger than 6 cm in greatest dimension and ENE(-)
	N2a	Metastasis in a single ipsilateral node larger than 3 cm but not larger than 6 cm in greatest dimension and ENE(-)
	N2b	Metastasis in multiple ipsilateral nodes, none larger than 6 cm in greatest dimension and ENE(-)
	N2c	Metastasis in bilateral or contralateral lymph nodes, none larger than 6 cm in greatest dimension and ENE(-)
N3		Metastasis in a lymph node larger than 6 cm in greatest dimension and ENE(-); or metastases in any node(s) with clinically overt ENE(+) (ENE_c)**
	N3a	Metastasis in a lymph node larger than 6 cm in greatest dimension and ENE(−)
	N3b	Metastasis in any node(s) with clinically overt ENE(+) (ENE_c)**

*Midline nodes are considered ipsilateral nodes.
**ENEc is defined as invasion of skin, infiltration of musculature, dense tethering or fixation to adjacent structures, or cranial nerve, brachial plexus, sympathetic trunk, or phrenic nerve invasion with dysfunction Note: A designation of "U" or "L" may be used for any N category to indicate metastasis above the lower border of the cricoid (U) or below the lower border of the cricoid (L). Similarly, clinical and pathological ENE should be recorded as ENE(−) or ENE(+).

Note: A designation of "U" or "L" may be used for any N category to indicate metastasis above the lower border of the cricoid (U) or below the lower border of the cricoid (L). Similarly, clinical and pathological ENE should be recorded as ENE(−) or ENE(+).

MS-1

The Discussion section has been updated to reflect the changes in the algorithm.

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Updates in version 1.2018 of the NCCN guidelines for head and neck cancers from version 2.2017

Global changes

The term "extracapsular spread" has been changed to "extranodal extension."

"Multimodality clinical trials" has been changed to "clinical trials."

"Lymphovascular invasion" has been changed to "vascular/lymphatic invasion."

For those with positive margins after resection, the adjuvant therapy option of re-resection has been revised to "re-resection if feasible," and the following footnote has been removed: "Consider re-resection to achieve negative margins, if feasible."

TEAM-1

Under multidisciplinary team

Seventh bullet revised: "Physical medicine and rehabilitation (including therapy for lymphedema of the neck)"

Twelfth bullet revised: "Diagnostic and interventional radiology"

Cancer of the lip

LIP-2

The following has been moved from the primary therapy algorithm to a footnote: "Elective neck dissection not recommended."

The following option and subsequent pathway have been removed: "Consider resection of primary ± sentinel lymph node (SLN) biopsy (category 2B)".

Following surgical resection, a new pathway has been added for those with perineural/vascular/ lymphatic invasion, and RT is the recommended adjuvant therapy.

LIP-3

Observation has been added as an adjuvant therapy option for patients with one positive node without adverse features.

For those with extranodal extension and/or positive margins, the adjuvant therapy option of re-resection has been revised to "re-resection if feasible (for positive margin only)" and the following footnote has been removed: "Consider re-resection to achieve negative margins, if feasible."

LIP-4

Following therapy with definitive RT or systemic therapy/RT, imaging recommendations have been revised: "FDG-PET/CT (preferred) of primary and neck or CT of neck (with contrast).

Cancer of the Oral Cavity

OR-2

First and second primary therapy options combined: "Resection of primary (preferred) ± ipsilateral (guided by tumor thickness) or bilateral (guided by location of primary) neck dissection or SLN biopsy".

Adjuvant therapy revised for those with extranodal extension ± positive margins: "Systemic therapy/RT (category 1)".

OR-3

Adjuvant therapy revised for those with extranodal extension ± positive margins: "Systemic therapy/RT (category 1) or RT".

For those with positive margins, the adjuvant therapy options have been revised to "Systemic therapy/RT (category 1) or re-resection if feasible and consider RT if negative margins ."

OR-A (1 of 2)

The following dose has been moved down, below concomitant boost accelerated RT: "66–70 Gy (2.0 Gy/ fraction; 6 fractions/wk accelerated)." (Also on ORPH-A, HYPO-A, GLOT-A, SUPRA-A, ETHM-A, MAXI-A, ADV-A)

Cancer of the oropharynx

ORPH-1

First bullet revised: "Tumor human papillomavirus (HPV) testing by p16 immunohistochemistry (IHC) required"

Fifth bullet revised: "FDG-PET/CT" and moved under "as clinically indicated"

New pathways have been included for p16- disease versus HPV-mediated (p16+) disease.

Footnote "g" added: "The clinical staging definitions take into consideration the new AJCC 8th edition staging for oropharynx cancer, while referencing the staging criteria previously used in clinical trials on the management of oropharynx cancer."

ORPH-2

The following primary treatment options has been revised:

"Transoral or open resection of primary ± neck dissection."

"For T1-T2, N1 only, RT + systemic therapy (category 2B for systemic therapy)."

"Consider" removed for "systemic therapy/RT" for positive margins and other risk features. (Also on ORPH-3/4)

Footnote removed: "The recommendations for patients at high risk with extranodal extension + positive margins are based on randomized studies involving patients for whom the HPV status of their tumors was not specified." (Also on ORPH-3/4)

ORPH-3

Footnote removed: When using concurrent systemic therapy/RT, the preferred agent is cisplatin (category 1).

See Principles of Systemic Therapy (CHEM-A). (Also on ORPH-4)

ORPHPV-1 through ORPHPV-3

Pages have been added with pathways for HPV-mediated (p16+) disease.

ORPH-A 1 of 2

The last line has been revised: "Either IMRT (preferred) or 3D conformal RT is recommended..." (Also on ORPH-A, 2 of 2)

ORPH-B

This page has been added, titled "Principles of p16 Testing for HPV-Mediated Oropharyngeal Cancer."

Cancer of the hypopharynx

HYPO-1

Under clinical stage, the first option revised: Amenable to larynx-preserving [conservation] surgery (Most T1, N0, and selected T2, N0); Second clinical staging pathway redefined: "T1-3, any N"; Footnote removed: "Anatomical imaging is also recommended."

HYPO-2

Second primary treatment option revised: "Surgery: Partial laryngopharyngectomy (open or endoscopic) + ipsilateral or bilateral neck dissection, + hemithyroidectomy, and pretracheal and ipsilateral paratracheal lymph node dissection".

HYPO-3

Primary treatment option revised: "Partial or total laryngopharyngectomy + neck dissection, thyroidectomy and pretracheal and ipsilateral paratracheal lymph node dissection".

HYPO-5

Primary treatment option revised: "Total laryngopharyngectomy + neck dissection + hemi- or total thyroidectomy, after ipsilateral or bilateral paratracheal lymph node dissection".

Cancer of the nasopharynx

NASO-1

Sixth bullet revised: "Imaging for distant metastases with FDG-PET/CT and/or chest CT with contrast.

NASO-2

Under primary treatment for T1, N1-3; T2-T4, any N, the category 3 has been removed from the option of induction chemotherapy followed by chemo/RT. (Also on CHEM-A, 1 of 5).

NASO-A

Last line revised: "Either IMRT (preferred) or 3D conformal RT is recommended for cancers of the nasopharynx to minimize dose to critical structures. Proton therapy can be considered when normal tissue constraints cannot be met by photon-based therapy."

Cancer of the glottic larynx

GLOT-1

Under workup, last bullet revised: " Pulmonary function evaluation for conservation surgery candidates."

GLOT-3

For N0,N1 disease after surgery, the following line has been added to the primary treatment options: "... and pretracheal and ipsilateral paratracheal lymph node dissection."

GLOT-4

After surgery, option revised: "Laryngectomy with thyroidectomy, ipsilateral, or bilateral neck dissection, and pretracheal and ipsilateral paratracheal lymph node dissection".

GLOT-5

For primary site < PR, "surgery" changed to "laryngectomy."

GLOT-6

The following line has been added to the primary treatment options for N0, N1, and N2-3 disease: "...and pretracheal and ipsilateral paratracheal lymph node dissection."

After primary treatment, pathways have been added to define the adjuvant therapy options for those with adverse features, and those with no adverse features.

Cancer of the supraglottic larynx

SUPRA-2

For positive margin, the adjuvant therapy option of re-resection has been revised to: "Re-resection if feasible, in highly selected patients".

Ethmoid sinus tumors

ETHM-2

Footnote "l" revised: "Adverse features include positive margins, high-grade lesions, and intracranial extension (See Discussion)."

ETHM-3

Following incomplete resection and no residual disease:

The following primary treatment option has been revised: "Surgery, if feasible".

The following adjuvant therapy option has been added for those after primary treatment with surgery: "Consider systemic therapy/RT (category 2B) if adverse features."

ETHM-A

Last line revised: "Either IMRT (preferred) or 3D conformal RT is recommended for maxillary sinus or paranasal/ethmoid sinus tumors to minimize dose to critical structures. Proton therapy can be considered when normal tissue constraints cannot be met by photon-based therapy." (Also on MAXI-A)

Maxillary Sinus Tumors

MAXI-1

Footnote "g" revised: "For sinonasal undifferentiated carcinoma (SNUC), small cell or sinonasal neuroendocrine carcinoma (SNEC) histologies, systemic therapy should be a part of the overall treatment. Consider a clinical trial and referral to a major medical center that specializes in these diseases." (Also on ETHM-1)

MAXI-3

Footnote removed: "For surgical resection, consider preoperative RT or preoperative systemic therapy/RT in select patients (category 2B)."

Very advanced head and neck cancer

ADV-2

For PS 0–1, the following primary treatment remains an option but has been removed from this page since it is included in the combination therapy options listed on CHEM-A (2 of 5): "Platinum + 5-FU + cetuximab (category 1)." (Also on ADV-4)

ADV-3

The primary treatment options have been revised for those with a locoregional recurrence without prior RT, if resectable: Surgery or Concurrent systemic therapy/RT or Induction chemotherapy (category 3) followed by RT or systemic therapy/RT.

Footnote "c" added: "When using concurrent systemic therapy/RT, the preferred agent is cisplatin (category 1). See Principles of Systemic Therapy (CHEM-A)."

ADV-A (1 of 2)

Line added to the chemoradiation section: "Data indicate that accelerated fractionation does not offer improved efficacy over conventional fractionation."

Ang K, Zhang Q, Wheeler RH, *et al*. A phase III trial (RTOG 0129) of two radiation-cisplatin regimens for head and neck carcinomas (HNC): Impact of radiation and cisplatin intensity on outcome [abstract]. J Clin Oncol 2010; 28(Suppl 15): Abstract 5507.

Bourhis J, Sire C, Graff P, *et al*. Concomitant chemoradiotherapy versus acceleration of radiotherapy with or without concomitant chemotherapy in locally advanced head and neck carcinoma (GORTEC 99-02): an open-label phase 3 randomised trial. Lancet Oncol 2012; 13: 145–153.

Footnote "2", line added: "Proton therapy can be considered when normal tissue constraints cannot be met by photon-based therapy. (Takiar V, Garden AS, Ma D, *et al*. Reirradiation of head and neck cancers with intensity modulated radiation therapy: Outcomes and analyses. Int J Radiat Oncol Biol Phys 2016; 95: 1117–1131.)"

Occult primary

OCC-1

Under workup, fourth bullet revised: "HPV, Epstein-Barr virus (EBV) testing for squamous cell or undifferentiated histology".

OCC-2

Definitive treatment revised following evaluation of level IV or V adenocarcinoma of neck node for intraclavicular primary: "Neck dissection if indicated ± adjuvant treatment if indicated (see OCC-4). Also, a link has been added to FOLL-A after treatment."

OCC-3

Indications have been revised for the following treatment options:

" Neck dissection (preferred for N1 disease, single node ≤3 cm)"; "RT for N1, single node ≤3 cm (category 2B)"; "Induction chemotherapy for N2-3 (category 3) followed by systemic therapy/RT or RT".

Salivary gland tumors

SALI-3

Cancer site descriptors have been revised:

Major salivary gland (parotid, submandibular, sublingual).

Minor salivary gland

Treatment option revised for majory salivary gland, clinical N0: "Surgery with complete resection of tumor ± neck dissection for high-grade and/or T3-4 tumors".

Treatment option revised for majory salivary gland, clinical N1: " Surgery + neck dissection",

Added "T3-4 tumors" to list of adverse features after complete resection of a major salivary gland tumor.

Adjuvant treatment options revised if adverse features after complete resection of a major salivary gland cancer: "Adjuvant RT or systemic therapy/RT (category 2B)".

SALI-4

The following recurrence therapy options have been added for those with distant metastases and PS 0-3:

"Androgen receptor therapy (ie. leuprolide, bicalutamide) if AR+; Trastuzumab if HER2+ (category 2B)"

Footnote "m" added: "Check androgen receptor (AR) status and HER2 status prior to treatment for distant metastases."

SALI-A

Last line added: "Proton therapy can be considered when normal tissue constraints cannot be met by photon-based therapy."

Footnote "2" revised: "Neutron therapy was historically considered a promising solution for unresectable salivary gland cancers, but this therapy is currently offered at only one center in the United States. Pfister DG, *et al*...".

SALI-A (continued)

Footnote "5" added: In general, the reirradiated population of head and neck cancer patients described in current literature represents a diverse but highly selected group of patients treated in centers where there is high

level of expertise and systems in place for managing acute and long-term toxicities. When the goal of treatment is curative and surgery is not an option, reirradiation strategies can be considered for patients who: develop locoregional failures or second primaries at ≥6 months after the initial radiotherapy; can receive additional doses of radiotherapy of at least 60 Gy; and can tolerate concurrent chemotherapy. Organs at risk for toxicity should be carefully analyzed through review of dose volume histograms, and consideration for acceptable doses should be made on the basis of time interval since original radiotherapy, anticipated volumes to be included, and patient's life expectancy. Proton therapy can be considered when normal tissue constraints cannot be met by photon-based therapy. (Takiar V, Garden AS, Ma D, *et al*. Reirradiation of head and neck cancers with intensity modulated radiation therapy: Outcomes and analyses. Int J Radiat Oncol Biol Phys 2016; 95: 1117–1131.)"

Mucosal melanoma

MM-1

Workup, fifth bullet revised: "Consider FDG-PET/CT or chest/abdominal/pelvic CT with contrast, and brain MRI (with and without contrast) to rule out metastatic disease".

MM-2

"Wide surgical resection" changed to "surgical resection." (Also on MM-3)

MM-3

Primary treatment options revised for stage III disease: "surgical resection, + neck dissection".

MM-4

Additional therapy revised after nodal dissection: "± RT to nodal bed for high-risk features".

Footnote "f" added: "High-risk, adverse features: >2 nodes, single node >3 cm, extranodal extension, recurrence in nodal basin after previous surgery."

MM-A

Last two lines added: "Either IMRT or 3D conformal RT is recommended. Proton therapy can be considered when normal tissue constraints cannot be met by photon-based therapy."

Follow-Up Recommendations

FOLL-A (1 of 2)

First line revised: "H&P exam (including a complete head and neck exam; and mirror and fiberoptic examination)".

Imaging recommendations have been grouped together.

FOLL-A (2 of 2)

Response after systemic therapy/RT or RT; First bullet revised under assess extent of disease or distant metastases: "FDG-PET/CTat minimum 12 wk"; Added after FDT-PET/CT: "If imaging is positive, CT of primary and neck or MRI with contrast".

Principles of surgery

SURG-A (3 of 8)

Added to fifth bullet: "If carcinoma in situ is present and if additional margins can be obtained that is the favored approach. Carcinoma in situ should not be considered an indication for concurrent postoperative chemoradiation."

SURG-A (5 of 8)

Under neck management, first bullet revised: "Tumor sites that frequently have bilateral lymphatic drainage (eg, base of tongue, palate, supraglottic larynx, hypopharynx, nasopharynx, deep pre-epiglottic…"

Line revised: "Patients with advanced lesions involving the anterior tongue, floor of the mouth, or lip alveolus that approximate or cross the midline should undergo contralateral selective/modified neck dissection as necessary to achieve adequate tumor resection."

SURG-A (6 of 8)

First bullet revised: "…Elective dissection depends on primary tumor extent and site.

For advanced glottic and hypopharyngeal cancers treated with primary surgery, a level VI dissection (including pretracheal lymph nodes, the delphian lymph node, and unilateral or bilateral paratracheal lymph nodes) and hemithyroidectomy to total thyroidectomy is appropriate. For primary subglottic tumors or glottic cancers with significant subglottic extension, a level VI dissection with unilateral or total thyroidectomy is considered appropriate based on the extent of the primary tumor. For example a T4a glottic tumor with extension through the cricothyroid membrane and subglottic extension should include a total thyroidectomy, and pretracheal and bilateral paratracheal lymph node dissection. Parathyroid glands should be preserved in situ or auto transplanted as indicated."

Radiation techniques

RAD-A (2 of 5)

Under IMRT, PBT and Fractionation, dosing revised in second sentence: The Simultaneous Integrated Boost (SIB) technique uses differential "dose painting" (66–72 Gy to gross disease; 44–63 Gy to subclinical disease) for each fraction…"

Last line added under proton beam therapy: "Proton therapy can be considered when normal tissue constraints cannot be met by photon-based therapy."

RAD-A (3 of 5)

First heading revised: Palliative Radiation 3D Conformal RT, IMRT, and SBRT.

Seventh bullet added under reirradiation: "For 3D conformal RT and IMRT: Standard dosing is 59.4–60 Gy at 1.8–2 Gy/fraction. Hyperfractionated schedule is 60 Gy at 1.2–1.5 Gy/fraction."

Principles of systemic therapy

CHEM-A (1 of 5)

Second bullet revised: "However, an improvement in overall survival with the incorporation of induction chemotherapy compared to proceeding directly to state-of-the-art concurrent chemoRT (cisplatin preferred, category 1) has not been established in randomized studies.

Third sub-bullet revised under induction/sequential chemotherapy for cancer of the Lip, Oral Cavity, Oropharynx, Hypopharynx, Glottic Larynx, Supraglottic Larynx, Ethmoid Sinus, Maxillary, Sinus, Occult Primary: "Following induction, agents used with concurrent chemoradiation typically include weekly carboplatin, weekly cisplatin (category 2B), or weekly cetuximab."

CHEM-A (2 of 5)

Gemcitabine/vinorelbine has been removed from the options for nasopharyngeal cancer.

Cisplatin/gemcitabine has been changed from a category 2A to a category 1 recommendation for recurrent, unresectable or metastatic nasopharyngeal cancer.

New headings have been added to identify the first-line therapy options and second-line/subsequent therapy options.

Pembrolizumab has been added as a category 2B, second-line therapy option for nasopharyngeal cancer, if previously treated, PD-L1-positive recurrent or metastatic disease.

CHEM-A (3 of 5) through CHEM-A (5 of 5)

References have been updated.

Principles of nutrition

NUTR-A (1 of 2)

New section added for pain management with the following bullet and references: Assess pain from oral mucositis and prescribe gabapentin or doxepin as clinically indicated.

Bar Ad V, Weinstein G, Dutta PR, *et al*. Gabapentin for the treatment of pain syndrome related to radiation-induced mucositis in patients with head and neck cancer treated with concurrent chemoradiotherapy. Cancer 2010; 116: 4206–4213.

Leenstra JL, Miller RC, Qin R, et al. Doxepin rinse versus placebo in the treatment of acute oral mucositis pain in patients receiving head and neck radiotherapy with or without chemotherapy: a phase III, randomized, double-blind trial (NCCTG-N09C6 [Alliance]). J Clin Oncol 2014; 32: 1571–1577.

Staging

ST-1

Staging tables have been updated to reflect the AJCC 8th Edition Cancer Staging System.

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