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热烈祝贺

Chinese-German Journal of Clinical Oncology (《中德临床肿瘤学杂志》) 被EMBASE和Index Copernicus收录

《中德临床肿瘤学杂志》通过严格评审，于2010年被EMBASE和Index Copernicus收录。

EMBASE是由Elsevier公司出品，Excerpta Medica（荷兰《医学文摘》）的在线版本。涵盖70个国家/地区出版的3800多种期刊，覆盖各种疾病和药物的信息。

Index Copernicus (波兰《哥白尼索引》) 是由Medical Science International (国际医学) 创办的医药学、生物学国际检索系统，以收集生物学、医药学内容为主。近年来逐步扩大收录的学科范围，同时收集数学、物理、化学、地学等科学信息，成为世界性门户。每年,《哥白尼索引》根据期刊"科学质量"、"编辑质量"、"国际影响力"、"按时发行"和"印刷质量"等评价标准对其收录期刊进行多参数的质量评价。

《中德临床肿瘤学杂志》进入EMBASE和Index Copernicus数据库，是对期刊整体水平的肯定。我们将以此为契机，不断开拓进取，努力提高期刊影响力，更好地为肿瘤学研究人员服务！

自创刊以来，本刊已先后被SpringerLink数据库，中信所科技核心数据库，中国期刊全文数据库、万方数据资源系统数字化期刊群、维普资讯网科技期刊数据库、中国学术期刊综合评价数据库、EMBASE、Index Copernicus等国内外重要检索系统收录。

在此，我们衷心感谢广大编委、作者、读者对本刊的大力支持，并欢迎国内外从事肿瘤学及其相关领域研究的科研工作者踊跃向本刊投递高质量的稿件。我们愿意竭尽所能为您服务，共同搭建一个与全世界科研工作者相互交流的平台，使您的科研事业更上一级台阶！

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Analysis of cancer pain in hospitals in Beijing, China

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Abstract ***Objective:*** This study aimed to survey the characteristics and treatments of cancer pain in Beijing hospitals, China. ***Methods:*** At 20:00 on December 22, 2009, there conducted a questionnaire survey in 2238 patients with malignant tumors of 26 hospitals in Beijing, and the survey results were statistically analyzed. ***Results:*** The 531 cases in 2238 patients had pained and 121 cases (22.79%) had outbreak pain with one week. At 20:00 on December 22, 2009, 199 cases (38%) in the above-mentioned 531 pain patients suffered the moderate to severe pain (pain scores ≥ 4). The number of pain (pain scores ≥ 4) patients in the consecutive three days from December 20 to December 22 were 150. ***Conclusion:*** In the 531 cancer pain patients of the surveyed hospitals, 38% of the patients were not satisfied with the pain control. Doctors believed that the main reason for pain not controlled was the non-standard treatment, but patients considered to be afraid of addiction.

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Key words cancer; pain; treatment; questionnaire

Pain is a common symptom of malignancies that compromise patients' quality of life. It was widely accepted by researchers at the Second Asia Pacific Symposium on Pain Control in February 2001, that pain management is one of the basic human rights of patients. Pain is also the fifth vital sign after body temperature, pulse rate, respiration rate, and blood pressure. A three-stage analgesic protocol for pain control has been recommended by the World Health Organization for over 20 years. Non-steroidal anti-inflammatory drugs and weak/strong opioids are options for pain management. The American National Comprehensive Cancer Network (NCCN) Practice Guidelines for Cancer Pain has rapidly circulated around China in the last 5 years. However, cancer pain management is still substandard. Recently, the department of oncology of a renowned hospital in Beijing, China, conducted a survey among hospitalized cancer patients and found that 21 of 168 patients endured pain, including 12 cases of mild pain, eight cases of moderate pain, and one case of severe pain. The patient with severe pain had suffered for 30 days, unrecognized by physicians or nurses. With these results from the survey, the Committee of Rehabilitation and Palliative Care of Beijing Anti-cancer Association organized oncologists from 26 hospitals in Beijing to conduct a joint survey on cancer pain control in hospitalized patients with malignancies on December 22, 2009.

Materials and methods

Subjects

At the appointed time, 2238 hospitalized patients with malignancies were surveyed through a self-administered questionnaire. Within the previous 7 days, 531 of 2238 patients had pain, 76 (14.3%) of the 531 patients were from a Level 3 hospital (the highest level in the hospital ranking system) and the other 455 (85.7%) were hospitalized in lower-level hospitals. Two hundred and forty three (45.8%) of the 531 patients were hospitalized in cancer specialty hospitals and the other 288 (54.2%) were in general hospitals. The hospitals included in the survey were presented in Table 1.

Methodology

The questionnaire consisted of questions regarding the level of hospital; whether the hospital was a specialty cancer hospital; whether the department was a cancer department; the demographics of the patients with cancer pain; the onset and duration of cancer pain; the clinical and pathological diagnosis of the primary cancer; the site, nature, cause and numerical rating scale (NRS) grade of cancer pain; drugs used for pain management; adverse events of pain management; and the cause of uncontrolled pain at the time of the survey.

Pain was evaluated using a NRS of 0 to 10 as recommended by NCCN Practice Guidelines for Cancer Pain as follows: 0, no pain; 1–3, mild pain; 4–6, moderate pain; and 7–10, severe pain.

Quality control

Oncologists conducting the survey were all attending physicians in the wards. They received systematic training in terms of the goal, significance, methodology and quality monitoring of this survey. They were able to accurately and objectively complete the questionnaire, and qualified to retrieve and collect information on pain during the previous week from patients with cancer pain. These physicians then instructed patients on the use of the questionnaire, the patients were allowed to complete it themselves. The physicians filled in the questionnaire if a patient was unable to write.

Table 1 Hospitals included in the survey

Hospitals	
307 Hospital of the People's Liberation Army	304 Hospital of the People's Liberation Army
Beijing Cancer Hospital	Beijing Fengtai Hospital
The General Hospital of the People's Liberation Army	Xiyuan Hospital of China Academy of Chinese Medical Sciences
Cancer Institute & Hospital of Chinese Academy of Medical Sciences	Beijing Shijitan Hospital
Beijing Sanhuan Hospital	Beijing Puxiang TCM Tumor Hospital
Peking Union Medical College Hospital	General Hospital of the Navy of the People's Liberation Army
Beijing Fuxing Hospital	Air Force General Hospital of the People's Liberation Army
Beijing Chest Hospital	Beijing Wangjing Hospital of China Academy of Chinese Medical Sciences
China-Japan Friendship Hospital	Beijing Zhongguanchun Hospital
Beijing University of Chinese Medicine's Third Hospital	Beijing Haiding Hospital
Beijing Chaoyang Hospital	309 Hospital of the People's Liberation Army
Military General Hospital of the Beijing People's Liberation Army	Beijing Shunyi Hospital
General Hospital of the Second Artillery Force of Chinese PLA	Beijing Cangping District Hospital

Table 2 Clinical characteristics of 531 patients with cancer pain

Characteristics	Patients		Characteristics	Patients	
	n	%		*n*	%
Gender			Primary cancer type		
Male	304	57.3	Lung cancer	185	34.8
Female	227	42.7	Breast cancer	55	10.4
Age (years)			Cancer of the large intestine	51	9.6
< 35	36	6.8	Gastric cancer	36	6.8
35–60	250	47.1	Nasopharyngeal cancer	26	4.9
> 60	245	46.1	Esophagus cancer	24	4.5
Hospital level			Pancreatic cancer	18	3.4
Third-level	76	14.3	Lymphoma	14	2.6
Lower-level	455	85.7	Liver cancer	12	2.3
Type of hospital			Bladder cancer	12	2.3
Cancer hospital	243	45.8	Kidney cancer	9	1.7
Comprehensive hospital	288	54.2	Cholangiocarcinoma	8	1.5
Metastasis/recurrence			Ovarian cancer	5	0.9
No	298	56.1	Vaginal carcinoma / Cervical carcinoma	5	0.9
Yes	233	43.9	Prostate cancer	4	0.8
Organs involved in metastasis or recurrence	84	15.8	Osteosarcoma	4	0.8
Bone	69	13.0	Multiple myeloma	4	0.8
Brain	9	1.7	Melanoma	3	0.6
Soft tissue	21	4.0	Thyroid cancer	3	0.6
Multiple	45	8.5	Primary site unknown	21	4.0
Others	5	0.9	Others	32	6.0

Table 3 Characteristics of pain in 531 patients with cancer pain

Characteristics	Patients		Characteristics	Patients	
	n	%		*n*	%
Site of pain			Nature of pain		
Somatic pain	283	53.3	Dull pain	313	58.9
Visceral pain	153	28.8	Burning pain	47	8.9
Neuropathic pain	45	8.5	Stabbing pain	81	15.3
Multiple	38	7.2	Electric shock-like pain	12	2.3
Others	12	2.3	Multiple	35	6.6
Duration of pain			Others	43	8.1
Unremitting pain	225	42.4	Cause of pain		
Intermittent pain	264	49.7	Directly cancer-related	380	71.7
Breakthrough pain	10	1.9	Cancer treatment-related	101	19.0
Multiple	25	4.7	Both	20	3.8
Others	7	1.3	Others	30	5.5

Statistical analysis

Data were input into the database using Excel 2000. Statistical analysis was conducted by using SAS 9.1 system. Measurement data were presented as mean ± standard deviation, while categorical data were expressed as number and percentage. A statistically significant difference was considered at $P < 0.05$.

Results

Clinical characteristics of cancer patients

A total of 2238 hospitalized patients with malignancies were surveyed through the self-administered questionnaire, and 531 reported pain within the previous 7 days. The average age of the patients with cancer pain was (57.8 ± 14.7) years. Twenty primary tumor sites were involved in these 531 patients, and mostly included lung cancer, breast cancer, cancer of the large intestine, gastric cancer, nasopharyngeal cancer, and esophageal cancer. Of these cancer patients 233 (43.9%) had metastasis/recurrence while the remainder (56.1%) had none. A summary of clinical characteristics of patients with cancer pain was presented in Table 2.

Table 4 Summary of analgesic use in 199 patients with NRS ≥ 4

Characteristics	Patients		Characteristics	Patients	
	n	%		*n*	%
Single/combination use			Common analgesics (not including single auxiliary medication)		
Single analgesics	126	63.3	OxyContin		
Multiple analgesics	24	12.1	Single	51	34.0
Single auxiliary medication (e.g. herbs)	12	6.0	Combination	12	8.0
No analgesic use	37	18.6	Oxycodone		
Opioids			Single	22	14.7
No	139	69.8	Combination	3	2.0
Yes	60	30.2	Fentanyl		
Immediate-/sustained-release analgesics (without single auxiliary medication)			Single	20	13.3
Immediate-release	52	34.7	Combination	8	5.3
Morphine	15	10.0	Bucinperazine		
Sustained-release	78	52.0	Single	10	6.7
Sustained-release + Sustained-release	1	0.6	Combination	3	2.0
Immediate-release + Sustained-release	19	12.7			

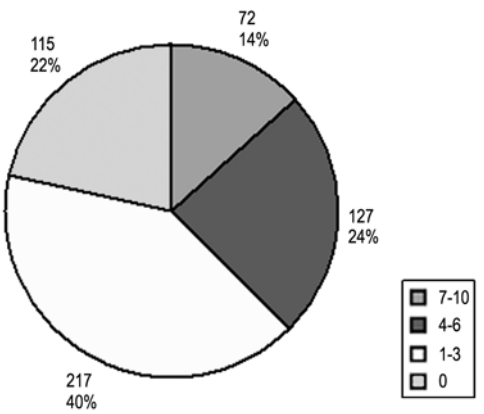

Fig. 1 Distribution of NRS for 531 patients with cancer at the time of the survey

Characteristics of pain in patients with cancer pain

The site, duration, nature and cause of pain were summarized in Table 3.

Pain management for patients with cancer pain

On December 22, the average NRS for 531 patients with cancer pain was 3.85 ± 2.26 (minimum: 0; maximum: 10). The distribution of NRS was presented in Fig. 1.

At the specific time of the survey, 331 (62.33%) patients with cancer pain received analgesics. Of the 531 patients, 199 (38%) endured moderate or severe pain (NRS ≥ 4), and 150 had moderate or severe pain (NRS ≥ 4) in the previous 3 days. Twelve patients also had NRS ≥ 4 after analgesic medication for 7 consecutive days. The analgesic use in the 199 patients with NRS ≥ 4 was summarized in Table 4.

The patients with pain medication presented with various degrees of treatment-related adverse events. The summary of adverse events for 336 patients with pain treatment was presented in Table 5.

Table 5 Summary of adverse events for 336 patients with pain treatment

Adverse events	Patients	
	n	%
Nausea	87	23.8
Vomiting	39	10.7
Constipation	119	32.5
Itching	9	2.5
Respiratory inhibition	14	3.8
Dizziness	35	9.6
Lethargy	30	8.2
Difficulty urinating	16	4.4
Others	17	4.6

Reasons for uncontrolled cancer pain according to physicians and patients

A total of 77 physicians and 104 patients commented on the reasons for uncontrolled cancer pain. Physicians considered that failed pain control mainly resulted from substandard treatment, while patients believed that their fear of drug addiction was the major cause of undertreatment of pain. A summary of the reasons for uncontrolled cancer pain was presented in Table 6.

Discussion

Pain is one of the most reported subjective symptoms by cancer patients. How to effectively alleviate pain and improve the patients' quality of life is a common concern to healthcare providers in clinical practice [1]. We conducted this survey to understand the current situation of cancer pain control in Beijing, China.

Table 6 Summary of reasons for uncontrolled cancer pain reported by 77 physicians and 104 patients

Characteristics	Number		Characteristics	Number	
	n	%		*n*	%
Physicians			Patients		
Lack of awareness of pain control	11	14.3	Fear of drug addiction	30	28.8
Substandard treatment	25	32.5	Economic burden	13	12.5
Undertreatment of adverse events	11	14.3	Irregular medications	21	20.2
Medications unavailable	1	1.3	Fear of causing inconvenience	13	12.5
Others	29	37.7	Others	27	26.0

At the survey time, 531 (62.33%) of 2238 patients with malignancies had suffered pain within the previous week. Of the 531 patients, 199 (38%) endured moderate or severe pain (NRS ≥ 4) on the day of the survey and 150 had moderate or severe pain (NRS ≥ 4) in the last 3 days, indicating that cancer pain is not managed well in many patients.

The 199 patients with uncontrolled pain (NRS ≥ 4) had been given analgesics on December 22, mainly OxyContin, oxycodone, fentanyl or bucinperazine. On the survey day, only 15 (10%) patients were given an infusion of immediate-release morphine. The results indicated that many physicians from the 26 hospitals did not comply with the guidelines for pain control in China and abroad.

Cancer patients, having need of pain treatment, are subject to pain control procedures. Their informed understanding of pain treatment, compliance with treatment and satisfaction are directly related to the efficacy of pain control. Results in the study found that fear of drug addiction accounted for 28.8% of the reasons for uncontrolled pain reported by patients, and that 20.2% of the patients did not receive timely medications, while 12.5% were reluctant to take medications owing to the possibility of causing inconvenience to family members or physicians.

Though the cancer patients may report different reasons for uncontrolled pain, they passively take medications. Compliance to pain medications often relies on recognition and understanding of treatment and therapies. Healthcare providers are responsible for and should be able to persuade patients to accept effective pain control methods. Eliminating patients' fears of analgesic addiction and enhancing publicity and education about pain control are the next steps to promote standard analgesic care in patients.

Among the reported reasons for uncontrolled cancer pain by physicians, 14.3% considered a lack of awareness of pain control; 32.5% substandard treatment; and 14.3%, undertreatment of adverse events. However, 1.3% also considered the unavailability of medications as a reason. We found that the traditional medical mode still had an impact on physicians' behavior. For a long time, physicians followed the cycle of patients' complaints, then treatment, then efficacy evaluation. For example, in the oncology department the cycle existed as cancer diagnosis, then standard chemotherapy or radiotherapy, then outpatient visits once every 3 weeks. In the department of surgery, the routine of disease diagnosis, surgery, then an outpatient visit at one month is an established routine. In the radiotherapy department, there is cancer diagnosis, radiotherapy planning, radiotherapy implementation, then an outpatient visit at 3 months all taking place consecutively. Simply put, when patients report complaints, physicians respond in a simple manner where no follow-up or modification of treatment scheme is considered. Thus the routine of complaints from patients and treatment from physicians followed by evaluation requires modification.

Cancer pain control has unique requirements. For moderate and severe cancer pain, no individualized dose can be established if no analgesics were administered previously in patients. Dose adjustment is required to be repeated within 1–2 days for pain management. The maximum interval between dose evaluations is only one hour and the minimum may be 15 minutes. Additionally, nausea, vomiting, dizziness, and constipation should be carefully monitored until the NRS is lowered to less than 4 without accompanying intolerable adverse events. This is a task of high intensity and heavy burden that cannot be accomplished without time-consuming or dedicated care.

Because of a high necessity, we suggest that physicians should change the old-fashioned concepts of cancer pain control to maintain compassion for patients and dedication to their work. Moderate and severe cancer pain should be rapidly and effectively managed as they are the equivalent of acute symptoms such as heart failure and bronchial asthma. Only when physicians include cancer pain in the clinical acute symptoms, can it be controlled rapidly, accomplishing the requirement of the NCCN guidelines for control within 24 h for severe pain and 48 h for moderate pain. Currently, physicians in China routinely adopt the protocol of an initial dose twice a day and dose evaluation every 2–3 days, which compares unfavorably with the international standard, and is possibly the root cause of the lack of control of cancer pain.

As cancer pain control for each patient is time-consuming and detailed, it needs massive medical input.

Most physicians with an overwhelming daily schedule are hardly able to meet the pain control needs of cancer patients. We suggest that a cancer pain control group, composed of a physician, a nurse, the patient and family members should monitor the pain control process.

Healthcare providers need to update the protocols of cancer pain control and take into account the following aspects. First, cancer pain should be evaluated in an appropriate manner. Evaluation of cancer pain is the prerequisite for pain control and determines the success of cancer pain treatment. Second, infusion of immediate-released opioids should be considered. For opioid-naïve patients, the process consists of an immediate-release phase followed by a controlled-release phase. Third, breakthrough pain control should be standardized. Fourth, refractory cancer pain should be treated in a standard manner. Fifth, education for patients and their family members should be emphasized. A new item in the NCCN Practice Guidelines for Cancer Pain highlights education for patients and their family members [2]. The patients and family members with good knowledge of pain control can help in cancer pain control, allowing improved efficacy of pain management.

Pain control is a complicated and delicate systematic process. Pain control for each patient may have both positive and negative aspects, but we hope the outcomes can be positive. This requires the concept of whole-process cancer pain control in healthcare providers. Compliance to NCCN Practice Guidelines for Cancer Pain to monitor health care services will also help standardize pain control procedures. We believe that a pain control group composed of a physician, a nurse, patient and family members will allow compassionate pain-free care for each cancer pain patient.

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The diagnostic evaluation of fine needle aspiration cytology of thyroid and its clinical application

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Abstract ***Objective:*** The aim of the study was to investigate the diagnostic value of fine needle aspiration cytology (FNAC) and its clinical application. ***Methods:*** From April 2009 to February 2011, thyroid FNAC were performed in a total of 186 patients with thyroid nodule or mass in our hospital and 78 of those 186 patients subsequently underwent thyroidectomy. The FNAC findings were compared with the results of the corresponding histological diagnosis. ***Results:*** The results of thyroid FNAC for 186 patients showed that, (1) 166 cases of benign lesions, the detection rate was 89.24% (166/186), including 96 cases of nodular colloid goiter (51.61%), 28 cases of simple colloid goiter (15.05%), 38 cases of Hashimoto's thyroiditis (HT) (20.43%) and 4 cases of thyroid adenoma (2.15%); (2) 4 cases of suspicious malignant lesion, the detection rate was 2.15% (4/186); (3) 16 cases of malignant tumor, the detection rate was 8.60% (16/186). Seventy eight patients including malignant (16), suspicious malignant (4), HT (20) and nodular colloid goiters (38) cases diagnosed by FNAC were performed operation with thyroidectomy and the postoperative histopathologic results showed that there were 2 cases HT combined thyroid papillary carcinoma in HT 20 cases by FNAC, 15 cases of thyroid papillary carcinoma and 1 case of follicular carcinoma in 16 cases of malignant tumor by FNAC and 4 case of thyroid papillary carcinoma in 4 cases of suspicious malignant by FNAC. ***Conclusion:*** Thyroid FNAC is a valuable and reliable method for the diagnosis of the thyroid nodules or mass or even most diffuse thyroid diseases. Diagnosis of HT and thyroid papillary carcinoma can be made by thyroid FNAC. There was larger hint value for nodular colloid goiter and simple colloid goiter according to thyroid FNAC.

Key words thyroid; fine needle aspiration cytology (FNAC); diagnostic value

Some clinical studies showed that there were great significance for confirming the nature of the tumor by fine needle aspiration cytology (FNAC), such as breast [1, 2], thyroid, etc. The FNAC of thyroid has been used in clinical diagnostic for more than 50 years. In the current study, we performed thyroid FNAC for total of 186 patients with thyroid nodules or mass and compared the results of FNAC with those results of histopathologic diagnosis results for 78 patients who subsequently underwent thyroidectomy from April 2009 to February 2011. The diagnostic value of FNAC was investigated for patients with thyroid nodules or mass.

Materials and methods

General materials

One hundred and eighty six of patients with thyroid nodules or mass at Clinic College of Medical School of Nanjing University, China, between April 2009 and February 2011 were obtained for this study. The gender ratio (male to female) was 1:5.46 (male: 28, female: 158); Age of the patients was ranged from 19 to 68 (average: 36 ± 0.6) years old; Enlarged thyroid gland of the degree I or larger or a palpable thyroid mass were observed in each of all 186 cases. Seventy eight of these patients subsequently underwent the thyroidectomy in our hospital, and the specimens obtained by the operation were performed for the histopathological evaluation. The rest of patients did not undergo the operation in our hospital.

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Collection of the thyroid FNAC specimen

The fine-needle puncture of the thyroid was performed by the experienced surgeon who determined the preoperative localizing and angle of puncture carefully based on the diagnosis techniques of palpation. The patient maintained sitting position and the operation portion of neck was exposed fully during the puncture procedure. After local skin antisepsis of neck, the operator fixed puncture side of thyroid or mass with left forefinger and middle finger, held a 20 mL glass syringes connected with a needle (size 8) by the right hand simultaneously, penetrated the syringes, which was left 5 mL air inside, into the sub-

cutaneous, made the negative pressure state by suction, and then punctured twice or three times quickly in different directions. After puncture, the negative pressure was released immediately and the needle was taken off, the specimens from the thyroid or mass by thyroid FNAC were spread evenly on the surface of the glass slides. Dried specimens were fixed with 95% alcohol, then applied for further HE staining. The part of puncture was pressed for 5 minutes after the operation.

Diagnostic standard of cytology

Satisfactory slides: there were at least six groups of thyroid epithelial cells (TECs), each group contained 15–20 TECs with a flake or follicular sample structure on each. Otherwise, the slides without enough cells were considered unsatisfied.

The satisfactory slides were divided into subgroups as benign, malignant, uncertainly/suspicious malignant.

Results

Results of thyroid FNAC

The results of thyroid FNAC for 186 patients showed that (1) Benign lesions: 166 cases, the detection rate was 89.24% (166/186), including 96 cases of nodular colloid goiter (51.61%), 28 cases of simple colloid goiter (15.05%), 38 cases of Hashimoto's thyroiditis (HT) (20.43%), and 4 cases of thyroid adenoma (2.15%); (2) 4 cases of suspicious malignant lesion, and the detection rate was 2.15% (4/186); (3) 16 cases of the malignant tumor, and the detection rate was 8.60% (16/186).

Comparison of the results between the thyroid FNAC and the corresponding histopathological diagnoses

Total of 78 patients, including 16 cases of malignant, 4 cases of suspicious malignant, 20 cases of HT and 38 cases of nodular colloid goiter diagnosed by FNAC, were performed with the thyroidectomy. The postoperative histopathologic results showed that two cases HT combined thyroid papillary carcinoma in 20 cases of HT by FNAC, 15 cases of thyroid papillary carcinoma and 1 case of follicular carcinoma in 16 cases of malignant tumor by FNAC and 4 case of thyroid papillary carcinoma in 4 cases of suspicious malignant by FNAC. There was none of false positive report in the thyroid FNAC group.

Cytology performance of common thyroid diseases

HT

The TECs and inflammatory cells were observed microscopically. Inflammatory cells were mainly as the lymphocytes, plasma cells, etc. The TEC was arranged as the piece of massive, enlarge and pleiomorphic, and the nuclear was oval and light dye. The acidophilic change of TEC (Hurthle cells) was the characteristic for this disease. Cytoplasm of TEC was rich and wide, HE dyeing it red. There were sporadic lymphocytes in the background, especially lymphocyte infiltration between TECs, as a phenomenon so called "lymphatic epithelial". It was found lack of colloid or absent of colloid.

Graves hyperthyroidism

There were a great number of TECs microscopically, shown the distribution of piece of massive, enlarged nuclear, loosen nuclear chromatin, and wide cytoplasm. There were a few lymphocytes. More or less red blood cells could be found in the background.

Nodular colloid goiter

Microscopically, cytology finding was closely related with the different periods of nodular colloid goiter and the subsequent changes such as bleeding, degeneration, necrosis and calcification, fibrosis. If no subsequent changes, the specimens of FANC were the mixture of a few TECs and colloid; the nodular colloid goiter usually had a large number of colloids at the stage of highly instauration. The hemosiderin cells could be shown.

Simple colloid goiter

Microscopically, a great number of colloids and a few TECs could be found in specimens from FNAC. TEC was small and the nuclear was small, round and dark dye.

Thyroid papillary carcinoma

Microscopically, a great number of TECs could be found in FNAC specimens, and the TECs were group lumps, distinct cell borders. The nuclear was overlap and crowded, and there were papillary architecture, ground-glass opacity of nuclear (Fig. 1), distinct nucleoli, nuclear grooves and intranuclear pseudoinclusions, and it was easy to see nucleolus. The psammoma body was visible sometimes, and one case mainly showed for large psammoma bodies in the current study (Fig. 2).

Follicular adenoma

Follicular adenoma had various types and shapes, TECs and colloids were seen mainly with thyroid FNAC specimens; TEC was scattered, and the small and round nuclear with dark dye.

Discussion

Incidence of thyroid nodules or mass was 4%–7% [3], and to determine the nature of the mass or nodules is directly related with treatment choice. It is difficult to differentiate between benign and malignant nodules with the clinical physical examination and the ultrasonography, isotope I^{131} and so on. The thyroid cells within the cells composition can be directly obtained by the thyroid FNAC. The nature of the nodules or mass can be clarified according to the shape and number of the cells in the thyroid nodules or mass. The FNAC is widely considered

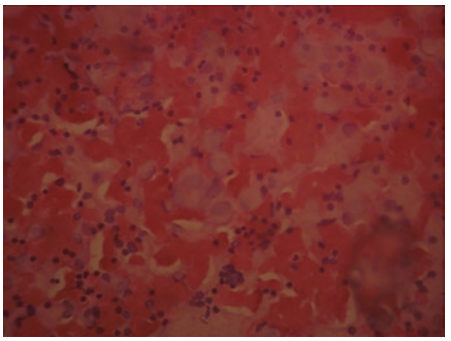

Fig. 1 Thyroid epithelial cells presented ground-glass opacity nuclear in thyroid papillary carcinoma (HE × 400)

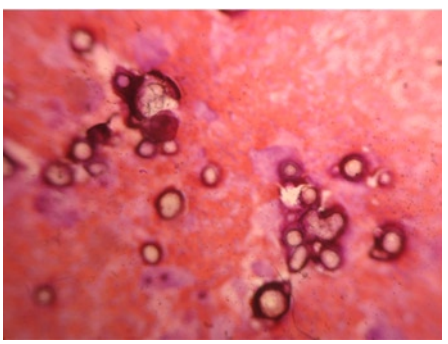

Fig. 2 Thyroid papillary carcinoma appeared a lot of psammoma bodies (HE × 40)

as the diagnostic technique of choice in the assessment of thyroid lesions. With the wide application of thyroid FNAC and the improvement of understanding of thyroid cell pathology, thyroid FNAC is no longer only for the identification of benign and malignant thyroid nodules to decide the requirement of surgical treatment [4]. Thyroid FNAC is also play an important guiding role in diagnosis, differential diagnosis and clinical treatment for some thyroid medicine diseases.

The finding of this study indicated that the diagnosis of the HT and thyroid papillary carcinoma could be made definitely by FNAC: (1) Diagnosis of HT: if the results of smear appear the phenomenon so called " lymphatic epithelial", HT should be highly suspected; If the smear with "lymphatic epithelial" phenomenon combined with eosinophilia TEC, piece of massive arrangement, nuclear pleomorphism and elliptic, light dye nuclear, the diagnosis of HT can be made. (2) Diagnosis of thyroid papillary carcinoma: if smear appear that there are a large number of TECs, with significant variability of its shape and size, the cells are group clumps, distinct cell borders, ground-glass opacity of nuclear, nuclear overlap and crowded, distinct nucleoli, nuclear grooves, and intranuclear pseudoinclusions seen easily, the diagnosis of thyroid papillary carcinoma can be made. If smear appear calcification small sample arrangement in concentric circles, namely psammoma body, thyroid papillary carcinoma can also be diagnosed, because psammoma body was hardly found in the benign thyroid disease up to date. It is very important to judge the psammoma body, and to avoid the misclassification of the irregular calcification as the psammoma body.

For four cases of suspicious malignant lesion by thyroid FNAC in our study, the patients underwent the surgery, and the postoperative pathologic results showed the thyroid papillary carcinoma for all of four cases. The possibility of malignant tumor is often suggested if cells form abnormalities can be found by FNAC.

Thyroid FNAC is also very helpful for the diagnosis of nodular colloid goiter and simple colloid goiter, e.g., the findings, such as more or less of the colloids, medium size of TECs, round and dark dye of nuclear with smear, often suggest nodular colloid goiter and simple colloid goiter. The published study [5] showed that FNAC was the "gold standard" for judgment of thyroid nodule and mass, and the diagnostic accuracy rate can amount to 95%.

In general, judge of benign and malignant with follicular tumors must be based on the capsule or vascular invasion or not, thus, thyroid FNAC has its limitations for the diagnosis of follicular carcinoma.

In summary, thyroid FNAC can be used in diagnosis of HT and thyroid papillary carcinoma, as well as provide the larger hint value for diagnosis of nodular colloid goiter and simple colloid goiter, based on its advantages, i.e., convenience and easy to operate, safety, less complications, rapid diagnosis and high accuracy [6], easy to be accepted by patients and doctors. Thyroid FNAC is a valuable and reliable method for the diagnosis of the thyroid nodules or mass or even most diffuse thyroid diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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Narcoanalysis of pneumonorestion with video-assisted thoracic surgery during one-lung ventilation*

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Abstract ***Objective:*** The aim of our study was to analyze the anesthesia of pneumonoresection in lung cancer patients with video-assisted thoracic surgery during one-lung ventilation. ***Methods:*** After fast-speed venous induced anesthesia, double-lumen bronchial catheter or endobronchial blocker tube were intubated in 551 patients, the position of double-lumen endobronchial tube or single lumen tube + endobronchial blocker tube was confirmed with fiber-optic bronchoscope after intubation. Interstitial positive pressure ventilation were used in all patients with video-assisted thoracic surgery (VATS) interstitial positive pressure ventilation, positive end expiratory pressure and continuous positive airway pressure in collapse lobers of lung were used in one lung ventilation, and ventilation parameters were adjusted necessarily. ***Results:*** 541 cases double-tubes bronchial catheter intubation and endobronchial blocker tube used by fiberscope were located very well. The level of S_pO_2, $P_{ET}CO_2$ could be maintained normal. Ten cases were forced to converse video-assisted thoracic surgery to thoracotomy because of 4 cases pulmonary adhesion, 4 cases severe pulmonary dysfunction hard to correct hyoxemia and 2 case abnormal anatomy respectively. ***Conclusion:*** Anesthesia key of video-assisted thoracic surgery is that double lung must separated completely. Effective management of one lung ventilation could make patients to pass perioperation smoothly. Long-time one lung ventilation such as pulmonary adhesion, severe pulmonary dysfunction should be considered to be relative contraindication.

Key words lung cancer; thoracoscopy; one lung ventilation; double-lumen endotracheal tube; bronchial blocker

The operation of pulmonary carcinoma with video-assisted thoracic surgery (VATS) is a new approach of minimally invasive surgery in recent years in clinical application, with the advantages of little operation trauma, little stress reaction, little destroy to physiologic thorax, little postoperative pain and recovery rapidly, especially in those lung cancer getting old and weak and inferiors cardio-pulmonary function which could not endure standard thoracotomy [1, 2]. Therefore the VATS is used more and more in clinical application. But there are some advance demands and particularities in anesthesia management with the surgery. The 551 surgery anesthesia cases with VATS were completed in our hospital so far. Ten cases were transferred into routine thoracotomy. The article is about the anesthesia analysis of pneumonoresection in lung cancer patients with video-assisted thoracic surgery during one-lung ventilation.

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Materials and methods

Materials

Five hundred and fifty-one pulmonary carcinoma patients with VATS were accrued from October 2006 to December 2011. Among them, 324 were male, 227 were female, age ranged from 22 to 81 years (average age, 57.5 ± 16.4 years); weight 39–89 kg, average weight (62.5 ± 9.8) kg; left pulmonary carcinoma 265 cases, right pulmonary carcinoma 286 cases; all patients were classified as ASA physical status I–IIII, pulmonary function test: 44 cases mild or moderate ventilation dysfunction and 6 cases severe airway obstruction.

Methods

Every patient received sodium phenobarbital 0.1 g and atropine 0.5 mg or scopolamine 0.3 mg, 30 min before entering the operation. All patients received general anesthesia. Oxygen inspiration and denitrification began and last for 5 min before anesthesia induction. Midazolam 0.05–0.1 mg/kg, propofol 1 mg/kg, fentanyl 2–4 μg/kg

and cisatracurium besylate 0.2 mg/kg were injected intravenously as induction drugs, lasted for 4 min. There were 290 cases used in left double-lumen tube, 201 cases in right double-lumen tube and 60 cases used with single lumen tube + endobronchial blocker tube. Methods: Robertshaw's double-lumen tube Fr 32–39 were inserted, usually in health side. The double-lumen tube was confirmed with fiberoptic bronchoscope (PENTAX FI-10BS, outside diameter 3.5 mm, Japan) after auscultation. The double-lumen tube was verified with fiberoptic bronchoscope, after lateral decubitus position was located again. When collapse lung expanded or oxygen saturation of blood (S_pO_2) dropped, there were possibilities of the shift of double-lumen tube. Double-lumen tube should be adjusted through fiberoptic bronchoscope promptly. And there was another way, an 8.0–8.5 mm (internal diameter) single lumen tube was intubated at first. The depth from fore-tooth to the tip of tube 22–23 cm. The tube was confirmed in endotracheal and located deliberately. Then endobronchial blocker tube and fiberoptic bronchoscope was directed through tracheal catheter simultaneously. The endobronchial blocker tube was advanced into the main-stem of operative side. And then ballonet of bronchial blocker tube was charged about 2–4 mL, the pressure of ballonet was test. After successful intubation, the air of ballonet was extracted and double lung ventilated in supine position. After later decubitus position was located, the endobronchial blocker tube was verified with fiberoptic bronchoscope (PENTAX FI-10BS, Japan). Propofol was transfused with target controlled infusion pump (target concentration 2–3 μg/mL). Fentanyl was administrated intermittently according to blood pressure (BP) and heart rate. Cisatracurium besylate 0.08–0.15 mg/kg·h was used to maintain muscular flaccidity. Minute ventilation, airway pressure, inspiratory: expiratory, BP, invasive blood pressure (IBP), electrocardiogram (ECG), S_pO_2, end-tidal carbon dioxide pressure ($P_{ET}CO_2$), temperature and urine volume were monitored continuously. Central venous pressure was monitored discontinuously, P_aCO_2, P_aO_2 were determined by blood gas analysis.

Mechanical ventilation of constant volume was maintained. Pulmonary ventilation: tidal volume (VT) 6–8 mL/kg, breathing rate 12–20 bpm, inspiratory: expiratory 1:2–3, The respiratory tract secretion was suctioned in time during the operation, when S_pO_2 was lower than 90% in one lung ventilation, positive end expiratory pressure (PEEP) 4–6 cm H_2O was given to health side lung, appropriate PEEP could increase lung volume and not raise shunt volume. 100% oxygen continuous positive airway pressure (CPAP) 5 cm H_2O was given to collapse lung in order to raise oxygen saturation in one lung ventilation. Patient-controlled intravenous analgesia pump were installed postoperatively.

Results

All patients' vital signs were maintained smoothly during anesthesia induction. All double-lumen tube were confirmed with fiberoptic bronchoscope after auscultation, operative side lung collapsed satisfactorily after one lung ventilation, operating field was exposed well. There were 32 cases which S_pO_2 was lower than 90% in one lung ventilation, it could reached normal level by above processing methods. But there were 4 cases because of lung general adhesive to thoracic wall, 4 cases could not endure long time hypoxemia and could meet the demand of the operation although one lung and two lung ventilation were implemented alternately. Two cases double-lumen tube located badly because of abnormal anatomy and one lung ventilation could implemented smoothly. Therefore all 10 cases were forced to converse VATS to thoracotomy. There was no any complication of anesthesia.

Discussion

The important anesthetic requirement of VATS was one lung ventilation, which ensured operative lateral lung collapse. Therefore that respiratory efficiency of the healthy side lung could endure one lung ventilation should be considered seriously at first [3]. Especially in pulmonary function was worse, patients could endure one lung ventilation was predicted, which should be considered contraindication of VATS [4]. Four cases could endure hyoxemia during one lung ventilation because of pulmonary dysfunction, so the patients were forced to converse VATS to thoracotomy. All patients' inspection result should be understood completely before operation, tumor position, size and endotracheal block level should be understood according to electronic brochoscopy, CT, MRI. Surgeons were communicated in time, operative program should be discussed, anesthesia could be prepared directly.

The use of double-lumen tube is the main technology of one lung ventilation at the present time because it costs less time and the operative side lung collapse rapidly. Under ordinary conditions, the use of double-lumen tube is more convenient, economical, effective than of endobronchial blocker tube. When double-lumen tube is hard to implement, endobronchial blocker tube shows its advantage in children, adolescents and dwarf, and can be a selective method. That the technology was use in children was reported [5, 6]. Moreover, under the circumstances of difficult airway, nasal intubation, tracheostoma, selective lung lobe blocked, endobronchial blocker tube is more superior to double-lumen tube [7–10]. In some cases, double-lumen tube has advantage over endobronchial. It is still the first selection of equipment for one lung ventilation such as sequential ventilation. Two cases right double-lu-

men tube was inserted as required. Because the open of right upper lobe was higher than normal by fiber-optic bronchoscope, the little cuff of double-lumen tube was above the carina, which could not make the left lung collapse well all along. Therefore the patients was decided to converse VATS to thoracotomy. Since 2009 the technology of endobronchial blocker tube was introduced, endobronchial blocker tube was used in all abnormal anatomy of tracheal bronchus, which can increase the indications of VATS and can avoid to converse VATS to thoracotomy because of locating badly of double-lumen tube [11].

The main reason of leading to hypoxemia during one lung ventilation is unbalance ratio of ventilation and blood flow at the present time, which effect factors include body position, anesthesia and hypoxic pulmonary vasoconstriction (HPV), especially HPV. Our experience showed in connection with pulmonary dysfunction, the emphasis of respiratory management during VATS are: (1) Select of narcotic: hypoxic pulmonary vasoconstriction is a kind of protective response by raising pulmonary vascular resistance when alveolar oxygen partial pressure lower, inhalation anesthetic inhibit hypoxic pulmonary vasoconstriction direct proportion to gas strength, destroy physical autoregulation. So inhalation anesthetic should be reduced during one lung ventilation; (2) Adjustment of right respiratory parameters: airway pressure go up and partial pressure of oxygen lower, so right respiratory parameters should be adjusted at first. High minute ventilation could lessen hypoxemia in favor of aero-phose, but increase pulmonary vascular resistance and make blood turn to collapse lung. Intrapulmonary shunt increase, which lead to aggravate oxygen-poor, but lower minute ventilation could lead to hypercapnia. We use lower tidal volume, fast breathing rate and high inspiratory/expiratory ration, which can help patients pass one lung ventilation [12]; (3) The respiratory tract secretion was suctioned in time during the operation to maintain respiratory tract smoothly. To lessen the possibilities of hypoxemia, oxygen concentration should be increased as far as possible, the change of the peak inspiratory pressures should be paid attention to; (4) Ventilation mode CPAP or PEEP could be used. S_pO_2, P_aO_2 is lower or drop badly, lower level PEEP in health side could raise alveolus volume at end expiration, make better to pulmonary functional residual capacity, keep the alveoli from collapsing and make it easier to expand the lungs, increase oxygenation time, so P_aO_2 could increase [13, 14]. The 0.49–0.98 kPa (5–10 cm H_2O) CPAP used in collapse lung did not effect the VATS. CPAP can protect and correct hypoxemia during one lung ventilation. Although the above measure were adopted, there are 4 cases that could not endure longer hypoxemia for pulmonary dysfunction. S_pO_2 began to lower 10–12 min later during one lung ventilation, drop gradually to 80% within 2 min. Anaesthetist exchanged the situation with surgeon, the operation had to pause 3–4 times for two lung ventilation. Because the operation of one pulmonary lobectomy or pulmonary lobectomy + lymph node dissection would need longer time for one lung ventilation, the 4 cases were decided to converse VATS to thoracotomy. If wedge-shape excision of lung or biopsy were implemented, which could pass the operation between one lung and two lung ventilation and in favor of recovery, even if pulmonary function was worse because of short operative time and lung collapse time.

Thoracoscope could not go into thorax for lung general adhesive to thoracic wall. Adhesion should be separated carefully, oozing of blood was more and more, chest X-ray film and CT were read carefully. In view of pleural thickening, medical history of pleuritis and pulmonary tuberculosis, the patients would hemorrhage more and could be stanched completely with VATS for long time, so the 4 cases were decided to converse VATS to thoracotomy. Thus health lung lobe should not be separated, which was easy to operate, could avoid exceptional affairs for more hemorrhage. In connection with these patients, medical history and film reading should be taken carefully, whether these patients could be operated with VATS should be considered carefully, which could lower patients' cost and save medical resource. It is necessary to converse VATS to thoracotomy, which can ensure surgery successful and patients safe, lower VATS complication. Along with more and more VATS, mature thoracoscope technology, which lead to less reason and lower ratio of conversing to thoracotomy.

The anesthesia key of thoracoscope is to ensure two lung separate well, operative side lung collapse completely, and operative field was exposed well in favor of operation. Pulmonary severe adhesion and severe pulmonary dysfunction should be considered relative contraindication of VATS within long time of one lung ventilation.

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Survivin ASODN targeted therapy in XWLC-05 cell transplanted nude mice*

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Abstract ***Objective:*** The aim of this study was to study the inhibiting effect of survivin mRNA on transplanted XWLC-05 tumor on nude mice. ***Methods:*** We established XWLC-05 transplanted nude mice model. 44 mice would be divided randomly into 4 groups: control group (blank), Lip group (simple liposome), survivin SODN group (transfected by sense oligonucleotide) and survivin ASODN group (transfected by antisense oligonucleotide). We would study general activities of nude mice in these 4 groups, measure the size of tumor and calculate the tumor inhabiting rate also. Pathological methods were applied in the analysis of the effect of different treatment on heart, kidney and liver of nude mice in these 4 groups. ***Results:*** Tumor grew slowly and size, weight of tumor was lower in survivin ASODN group when compared with that of others. Nude mice of survivin ASODN group showed lower growth index and tumor inhabiting rate was significantly higher than that of other groups ($P < 0.05$). Transplanted tumor on nude mice in control group (blank), Lip group, and survivin SODN group grew bigger as time passed and there was no significance among them ($P > 0.05$). We found a great deal of tumor cell necrosis in survivin ASODN group. No death of nude mice was observed in all 4 groups and we did not found obvious lesion in vital organs. ***Conclusion:*** Survivin ASDON could be used for the inhibitation of subcutaneously transplanted tumor in nude mice without obvious lesion in vital organs.

Key words survivin; antisense oligomucleotide; XWLC-05; animal experiment

Recently survivin discovered was belonged to inhibitor of apoptosis protein (IAP) family and became a research hotspot [1, 2] for scientists both in and out of China because of its special distribution characteristics in human body. Survivin can boost cell proliferation and inhibit necrosis. Survivin ASDON can significantly inhabit tumor cell proliferation in many kinds of tumor *in vitro*. However, rare report about its inhibiting function on tumor cells can be found. Our research would firstly establish XWLC-05 Xuanwei adenocarcinoma cell transplanted nude mice model by cell subcutaneous vaccination. Best tumor inhibiting concentration 600 nmol/L survivin ASODN was selected and injected multipoint in subcutaneous tumor. The growth of lung adenocarcinoma transplanted tumor in nude mice and survival of nude mice would be observed. We would evaluate the practical meaning of survivin ASODN used *in vivo*.

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Materials and methods

Composition of survivin ASODN and main reagents

According to relative literature [3], specially designed sequence was composed based on the sequence of Survivin mRNA 232–251 bp. The sequence of antisense oligonucleotide (ASODN) was 5-CCCAGCCTTCCAGCTCCTTG-3' and the sequence of sense oligonucleotide (SODN) was 5'-CAAGGAGCTGGAAGGCTGGG-3'. Both ends of these 2 kinds of sequence were base-modified by sulfur to avoid being degenerated by nuclease. We found out that survivin shared no homology with other human gene by compute based retrieval. Sequences above were synthesized by Shanghai Biotechnology Co. (China). RPMI1640, which was used for cell cultivation, were purchased from Gibco Co. (USA) and Calf serum was purchased from Hangzhou Sijiqing Biological Engineering Materials Co.

Ltd. (China).

Cell culture

Xuanwei lung adenocarcinoma cell line XWLC-05 was supplied by Medical Research Center of The First Affiliated Hospital of Kunmig Medical University. Cell line was preserved in refrigerator with temperature of -80 centigrade and was quickly thaw with temperature of 37 centigrade. Tube containing these cells was water-bathed under temperature of 37 centigrade and rang continuously so that all cells would be melted within 1 min. Then all cells would be transferred into centrifuge tube and 10 mL diluted RPMI1640 was added in. After 10 min in centrifugal machine (1000 r/min), we discarded supernatant solution and added RPMI1640 (concentration: 20%) into the tube. Cells were blowed and flapped softly for balanced mixing. Then all these cells were transferred in culture bottle and put in incubator with temperature of 37 centigrade, 5% CO_2 and saturated humidity. We selected passaged and logarithmic phased cells for next experiment.

The establishment of nude mice model

We bought nude mice BALB/c. All mice were about 4 weeks older or 6 weeks older with weight ranging from 18 g to 22 g. Balanced gender ratio was also ensured. All nude mice were fed under condition of specific pathogen free (SPF) and could eat and drink at will. XWLC-05 cells were conventionally cultivated for 1 month long. A great deal of cells would be collected on the same day we transplanted cells into nude mice. The amount of cell was 2.0×10^6 (about 0.5 mL cell solution), we injected these cells in the front side of left axillary of nude mice subcutaneously.

Group of experiment and injection of survivin ASODN

Nude mice would be put into experiment when the diameter of subcutaneously transplanted tumor measuring between 8 mm and 10 mm. We would select 44 nude mice randomly and divide them into 4 groups randomly. Nude mice in control group would be treated with normal saline. Nude mice in Lip group would be treated with certain amount (the same as survivin SODN group and survivin ASODN group) of liposome solution. Nude mice in survivin SODN group would be treated with composite of survivin SODN and liposome (600 nmol/L) and nude mice in survivin ASODN group would be treated with composite of survivin ASODN and liposome (600 nmol/L). Certain type of solution would be multipoint injected one time in transplanted tumor every 48 h according to different groups. We would inject 5 times in total.

Measuring size, weight of transplanted tumor and calculating tumor inhibiting rate

Before every injection,the longest diameter and the shortest one of tumor should be measured by vernier caliper. Every nude mouse should be weighted at the same time. Two weeks after the last injection, all nude mice should be put to death by dislocated broken neck method. We calculated the volume of tumor according to formula $V = ab^2/2$. Then tumor growth index would be calculated according to formula $V_{\text{before treatment}} - V_{\text{after treatment}} / V_{\text{before treatment}}$. The weight of tumor would be measured in order to calculate tumor inhibiting rate according to formula $(W_{\text{group control}} - W_{\text{group X}}) / W_{\text{group control}}$ (group X stand for the other 3 groups excluding control group).

General activity observation and pathological analysis

We would observe general activities including eating, drinking, pooping and emotion of nude mice. Heart, liver and kidney were processed by 4% paraformaldehyde for pathological analysis after death.

Statistical analysis

All data would be showed as $\chi \pm s$ and processed by SPSS12.0. F test would be applied for analysis between every 2 groups ($\alpha = 0.05$). If $P < 0.05$, we could consider the difference was significant. If $P < 0.01$, we could think the difference was extremely significant.

Results

General conditions of nude mice in 4 groups

Nude mice in 4 groups were all alive with normal general activities. Nothing obviously uncommon was found. We did not found obvious signs of necrosis and lesion of heart, liver and kidney.

The size of tumor and its growth index

In survivin ASODN group, tumor grew very slowly. Tumor in some nude mice in this group even stop growing and vanished. After total 5 times treatment, the tumor size of nude mice in survivin ASODN group was obviously smaller than that of other groups. However, tumor size of nude mice in other 3 groups were similar to each other. Tumor growth index of surviving ASODN group (2.81 ± 0.31) was significantly lower than that of other 3 groups (control group: 5.66 ± 0.29, Lip group: 6.37 ± 0.27, survivin SODN group: 5.89 ± 0.39; Fig. 1).

Tumor weight of these 4 groups and tumor inhibiting rate

Two weeks after the last injection, we put all nude mice to death by dislocated broken neck method and tumor weight would be measured. We found out tumor weight of nude mice in survivin ASODN group was (1.13 ± 0.08) g,

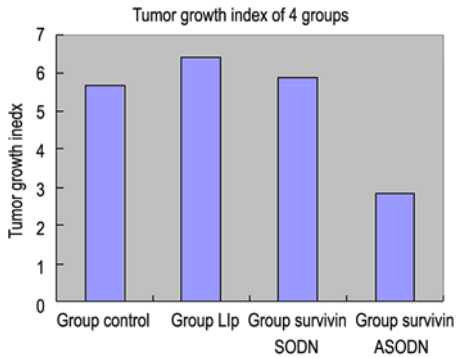

Fig. 1 Tumor growth index of 4 groups

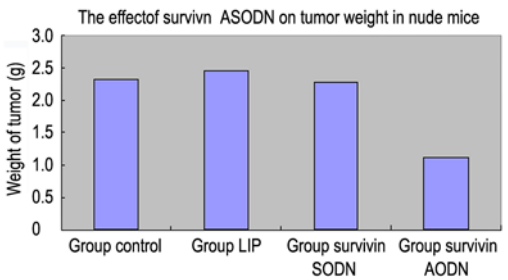

Fig. 2 The effect of survivin ASODN on tumor weight in nude mice

which was significantly lower than that of other 3 groups (control group: 2.31 ± 0.11, Lip group: 2.46 ± 0.09, survivin SODN group: 2.28 ± 0.08; Fig. 2). Tumor inhibiting rate of survivin ASODN group was (3.53 ± 2.46) %.

The effect of survivin ASODN transfection on tumor tissue of nude mice

We found that tumor cell could be both large and small with rich cytoplasm in the 3 groups excluding survivin ASODN group. Nuclear could be big, dark staining and pathologically divided. Sometime multiple nuclear mega cancer cell could be observed also. Though cancer cell of survivin ASODN group shared similarities with these 3 groups, we should not ignore that a great deal of cell necrosis, hyperplasia of connective tissue and obvious invasiveness of inflammatory cell.

Discussion

We can know from previous study survivin ASODN can affect survivin gene expression, inhibit the growth of Xuanwei lung adenocarcinoma cell and induce its apoptosis. So lung adenocarcinoma can be inhibited [4]. Experiment *in vitro* is totally different from the real in human body because of cell differentiation and totally different environment. So experiment *in vitro* can not be objective in evaluating treatment for certain disease and experiment practiced on human-disease animal model are significantly meaningful. In our study, we applied antisense technology. Specially designed sequence was composed based on the sequence of survivin mRNA 232–251 bp and base-mudified by sulfur. Then this processed sequence would be covered by liposome and finally injected multipoint into subcutaneous tumor in nude mice. Our study showed that tumor growth index (2.81 ± 0.31) of survivin ASODN group was significantly lower than that of other 3 groups and tumor in some of them stopped growing. Even tumor of 2 nude mice in this group vanished. We put all nude mice to death as scheduled and weighed all tumor tissue. We found that tumor weight (1.13 ± 0.08) of survivin ASODN group was significantly lower than that of other 3 groups and its tumor inhibiting rate was higher. More necrosis and apoptosis could be observed in tumor tissue of survivin ASODN group under microscope. These findings suggest us survivin ASODN can inhibit Xuanwei lung adenocarcinoma cell XWLC-05 significantly in nude mice subcutaneous tumor model. According to our experiment and relative literature [5], we guess survivin ASDON may get rid of the suppression effect of survivin in cancer cell so that more cells were inclined to apoptosis and fewer cells proliferated.

All nude mice were alive with normal condition before put to death. Pathological analysis showed no obvious signs of lesion in heart, liver and kidney. This suggests us it is safe to use liposome transfection and this may because survivn only specially expresses in tumor tissue [5]. This can also be strong support of target therapy of survivin ASODN.

Subcutaneously cultivated tumor of Xuanwei lung adenocarcinoma was obviously inhibited by survivin ASODN without apparent side effect. This suggests us antisense technology applied in Xuanwei lung adenocarcinoma is safe and feasible [6]. We hope our study can support future clinical treatment for lung adenocarcinoma and also be some guide for experiment.

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Palliative chemotherapy followed by consolidation radiotherapy in patients with advanced and metastatic non-small cell lung cancer not suitable for radical treatment

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Abstract ***Objective:*** This is a retrospective study to assess the effectiveness of consolidation radiotherapy (CRT) following palliative chemotherapy in patients with metastatic or locally advanced non-small cell lung cancer (NSCLC) who are not suitable for radical treatment. ***Methods:*** This study involved retrospective analysis of a prospective database of Northampton Oncology Centre from January 2005 to December 2010, 63 patients with advanced/metastatic NSCLC treated at the oncology centre were enrolled. Patients were either treated with high dose (39/36 Gy / 13-12 fractions, group 1) or low dose (20 Gy / 5 fractions, group 2) CRT or those were not offered any CRT (group 3). ***Results:*** There was no significant difference between the three groups as regard age, sex, performance status, comorbidities or chemotherapy given. However there was a statistically significant difference as regard the stage $P = 0.009$ with more stage IV patients at group II and III compared to group I. The mean survival for the three groups was 27 months, 14 months &15 months, respectively. There was a statistically significant improvement of survival in patients treated with high dose palliative CRT compared to the other two groups ($P = 0.006$). In multivariate analysis only the radiotherapy dose remains as the only statistical significant factor affecting the survival with hazard ratio 0.372 and confidence interval (0.147–0.726). ***Conclusion:*** Despite the limitation of our retrospective study, it is worth considering CRT approach for patients with advanced and metastatic NSCLC – not suitable for radical treatment – who have not progressed on chemotherapy.

Key words consolidation radiotherapy; non-small cell lung cancer; radiotherapy dose

Lung cancer is the leading cause of cancer related deaths in Western countries, with non-small cell lung cancer (NSCLC) accounting for more than 85% of primary lung cancers [1].

A minority of patients with unresectable non-small cell lung cancer whose lesions are confined to the thorax are selected for immediate, radical radiotherapy aimed at a cure or prolonging survival. For the remainder, however, advanced disease within the chest, the presence of distant metastases, or poor performance status preclude such potentially curative treatment [2].

Many patients with metastatic lung cancer (LC), and selected patients with locally advanced disease, are routinely treated with thoracic radiotherapy with palliative intent to relieve tumour-related symptoms (haemoptysis, bronchial obstruction, cough, shortness of breath, and chest pain) and improve health-related quality of life [3].

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Patients who usually require palliative radiotherapy upfront are suffering from symptoms that need faster radiotherapy intervention rather than waiting to see a response with palliative chemotherapy.

Cytotoxic chemotherapy is the mainstay of management in advanced NSCLC with response rates of 20%–40% and a median survival time of 7–10 months [4].

For patients with advanced NSCLC, we have moved from a situation of one size fits all to the dawn of individualized cancer therapy [5].

Despite the increased research in use of new chemotherapy drugs as well as biological agents, little has been done – as far as we know – to explore the position of palliative radiotherapy in the management plan for those patients, especially the concept of consolidation radiotherapy following chemotherapy.

In our work we looked at retrospective groups of patients who had been treated with palliative radiotherapy immediately after the end of chemotherapy treatment

and compared different fractionated regimens and also compared these to those patients who have been offered delayed radiotherapy.

Patients and methods

This study involved retrospective analysis of a prospective database of Northampton Oncology Centre from January 2005 to December 2010, 63 patients with advanced/metastatic NSCLC treated at the oncology centre were enrolled. The selected patients for analysis fulfilled the following criteria: (1) Patients are not candidates for radical treatment; (2) At least one cycle of palliative chemotherapy was administered with either stable disease or partial response; (3) No radiotherapy given prior to chemotherapy.

Patients were categorized into three groups: Group I: Patients who were offered high dose (39/36 Gy / 13-12 fractions) consolidation radiotherapy (radiotherapy given straight after the last cycle of chemotherapy); Group II: Patients who were offered low dose (20 Gy / 5 fractions) consolidation radiotherapy; Group III: Patients who were not offered any consolidation radiotherapy.

The decision to offer patients consolidation radiotherapy was mainly consultant driven as one oncologist adopts this approach; the other two consultants in the centre did not use it. All the patients and tumours' characteristics were extracted and analyzed.

Statistical analysis

Life tables and the log-rank (Kaplan Meier) test were used to test for significance of difference in survival in different treatment groups. Cox regression was used to test the effect of other risk factors on survival. Using backward stepwise Cox regression, only consolidation therapy remains in the last step model. Using forward stepwise Cox regression, only a number of fractions were accepted in the significant model. As the number of fractions is parallel to consolidation therapy, it was excluded from other risk factors included in the Cox regression model using the enter method. The *P* value was considered significant if less than 0.05. These tests were run on an IBM compatible personal computer using the Statistical Package for Social scientists (SPSS) for windows version 17 (SPSS Inc., Chicago, IL, USA).

Results

Twenty two patients were in group I, while group II included 19 patients and group III has 22 patients. Patients and tumour characteristics were presented in Table 1.

In group I, six patients received 39 Gy / 13 fractions and sixteen patients received 36 Gy /12 fractions, while in group II all patients received 20 Gy /5 fractions. Patients in all groups received 4 cycles of palliative chemotherapy on average.

Table 1 Patients and tumors' characteristics

	Group I	Group II	Group III
Age (years)	61	61	60
Males/females	15/7	12/7	10/12
PS			
0	6	3	4
1	8	10	15
2	8	6	3
Comorbidities			
1	13	7	14
2	6	8	5
3	3	4	3
Stage			
II	1	0	0
IIIA	5	0	1
IIIB	9	4	3
IV	7	15	18
Histology			
Adenocarcinoma	7	7	11
Squamous CC	9	4	10
Non-specified NSCLC	6	8	0
Chemotherapy			
Received 1st line	22	19	22
Received 2nd line	7	4	8
Received 3rd line	1	1	1

There was no significant difference between the three groups as regard age, sex, performance status, comorbidities or chemotherapy given. However, there was a statistically significant difference as regard to the stage ($P = 0.009$) with more stage IV patients at group II and III compared to group I.

The mean survival for the three groups was 27 months, 14 months & 15 months respectively, while the median survival was 21 months, 12 months & 14 months respectively. Survival curves are shown in Fig. 1.

There was a statistically significant improvement of survival in patients treated with high dose palliative consolidation radiotherapy compared to the other two groups ($P = 0.006$).

In multivariate analysis, only the radiotherapy dose remains as the only statistical significant factor affecting the survival with hazard ratio 0.372 and confidence interval (0.147–0.726) (Table 2). There was not enough data on the database to comment on the quality of life in the three groups.

Discussion

In lung cancer, the most commonly accepted symptomatic treatment consisted of palliative radiotherapy. With

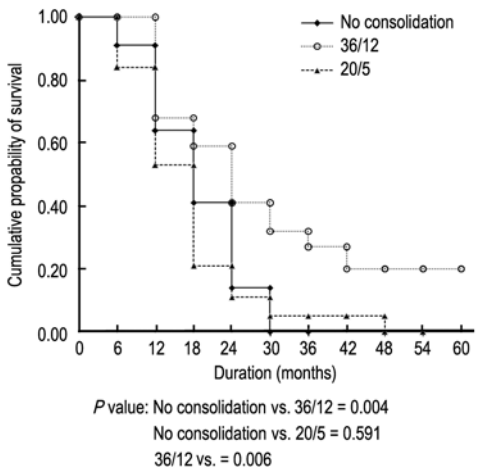

Fig. 1 Survival in studied groups

palliation as the aim, most patients should be treated with short courses of one or two fractions [6]. Various randomized trials and meta-analysis has extensively addressed the issue of radiotherapy dose and fractionation [6–20].

They all concluded that no significant differences were observed for specific symptom-control end points across all of the trials comparing low versus high radiotherapy, although improvement in survival favored high dose regimens.

Other six single-arm studies have confirmed symptom palliation after hypo-fractionated radiotherapy in patients with non-small cell lung cancer [21–26].

Our study has targeted selected groups of patients who have received at least more than one cycle of chemotherapy – without evidence of progression – followed by either consolidation radiotherapy in different fractionations or delayed radiotherapy on progression. This means that patients in our study were not suffering from significant local chest symptoms that necessitated upfront palliative radiotherapy and neither did they present with known brain metastasis.

The Norwegian Lung Cancer Study Group [27] in their randomized trail has concluded that non-symptomatic patients had significantly more favorable survival when compared to symptomatic patients with a median survival of 11.8 versus 6.0 months ($P < 0.0001$), respectively.

In our study, there was no survival benefit from consolidation radiotherapy with radiation dose 20 Gy/ 5 fractions, which highlights the importance of radiation dose in consolidation.

There is established evidence of survival benefit of a higher dose (HD) of radiotherapy as highlighted by the systematic review carried out by Fairchild *et al*, [7] where in the 13 analyzed trials, a statistically significant survival advantage was found for HD palliative radiotherapy, with 26.5% (420 of 1586) alive versus 21.7% (350 of 1613) at 2 years ($P = 0.002$). Sensitivity analysis suggests this survival improvement was seen with 35 Gy_{10} BED schedules compared with LDs. Overall survival at 2 years was reported by 10 trials, comprising 1,376 HD patients and 1,409 LD patients. A total of 8.1% were alive at 2 years after being treated with HD RT versus 6.7% treated with LD, with an OR of 0.82 (95% CI, 0.63 to 1.07; $P = 0.84$).

The Cochrane review in 2005 and 2009 has also addressed the radiation dose and fractionation questions with similar outcomes and acknowledged that in the future, large trials comparing different RT regimens may be difficult to set up because of the increasing use of systemic chemotherapy. The reviewer also recommended that trials looking at how best to integrate these two modalities, particularly in good PS patients, need to be carried out [28].

The National institute has issued guidance in 2005 and 2011 recommending that a high dose should be offered where the aim is to substantially reduce the size of the

Table 2 Multivariate Cox regression of survival on consolidation therapy and different risk factors

	Partial R	SE of partial R	Wald χ^2	*P*	Hazard ratio	95.0% CI for Hazard ratio	
						Lower	Upper
Consolidation			10.379	0.006			
Consolidation (36/12)	–1.118	0.407	7.553	0.006	0.327	0.147	0.726
Consolidation (20/5)	0.122	0.359	0.116	0.733	1.130	0.559	2.283
Sex (Male)	–0.007	0.290	0.001	0.980	0.993	0.563	1.752
PS	0.126	0.196	0.411	0.521	1.134	0.772	1.666
Hisotology			0.401	0.818			
Hisotology (Scc)	–.028	0.423	0.005	0.946	0.972	0.424	2.227
Hisotology (Adeno)	–.214	0.388	0.306	0.580	0.807	0.378	1.725
Stage	–.246	0.234	1.109	0.292	0.782	0.494	1.236
Comorbidities			1.363	0.506			
Comorbidities (Average/mild)	0.391	0.335	1.362	0.243	1.478	0.767	2.849
Comorbidities (Moderate)	0.172	0.404	0.181	0.671	1.187	0.538	2.623

SE: standard error; R: regression coefficient

cancer [29].

The recent ASTRO guidelines [30] has also advised that patients with good performance status may benefit from higher-dose/fractionation EBRT palliation (30 Gy / 10 fraction equivalent or greater).

The other question that also has been addressed before, but without much in the context of randomized control trial, is the timing of palliative radiotherapy in relation to chemotherapy.

In the MRC trial, addressing immediate versus delayed palliative thoracic radiotherapy in patients with unresectable locally advanced nonsmall cell lung cancer and minimal thoracic symptoms [31], they found that no persuasive evidence was found to indicate that giving immediate palliative thoracic radiotherapy improves symptom control, quality of life, or survival when compared with delaying until symptoms require treatment.

However, in this trial only short courses of radiotherapy were allowed (17 Gy / 2 fractions or 10 Gy single). It also has to be noted that none of those patients had been offered upfront chemotherapy.

We knew that response to first line chemotherapy is an important prognostic factor in this group of patients [32] and this is why we only offered consolidation radiotherapy to those who achieved at least, stable disease following chemotherapy.

In our study we endorsed the consolidation radiotherapy approach, which means radiotherapy given straight after the end of chemotherapy.

Recently, It has also been reported in a small trial looking at 20 patients with stage III NSCL treated with induction chemotherapy followed by radical radiotherapy that deferring radiotherapy after induction chemotherapy by more than 21 days has produced greater increases in percent volume change ($P = 0.002$) and percent diameter ($P = 0.055$) than lesser delays [33].

Conclusion

Despite the limitation of our retrospective small study, it is worth considering the consolidation radiotherapy approach for patients with advanced and metastatic non-small cell lung cancer – not suitable for radical treatment- who have not progressed on chemotherapy. A radiation dose of at least 36 Gy should be attempted in this group of patients. A national randomized trial is recommended.

Conflict of interest

The authors have no financial or personal relationship that could inappropriately influence/bias this work.

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Expression of proto-oncogene Fra-1 in human neoplastic breast tissues

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Abstract ***Objective:*** Invasion and metastasis are the most significant and intrinsic biological characteristics of cancers, also which are main factors of malignant tumor causing treatment failure and death. Recent studies have found that Fra-1 plays an important role on cell migration, invasion, and maintaining malignant phenotype of transformed cells. But there are few studies about the expression and location of Fra-1 in breast tissues and cells being reported .This study just aims to discuss the expression and location of transcription factor Fra-1 in benign and malignant human breast tissues. ***Methods:*** The expression of Fra-1 was investigated by immunohistochemistry in neoplastic breast diseases ranging from benign fibroadenoma to very aggressive undifferentiated carcinoma. The correlations of Fra-1 expression with other indicators of breast carcinoma prognosis (ER, PR and ErbB2 receptors) were analyzed. ***Results:*** All neoplastic breast tissues, either benign or malignant breast tissues, were nuclear immunoreactive for Fra-1-recognizing antibody. In 85% of benign tumors (17/20), the immunoreactive for Fra-1-recognizing antibody as exclusively restricted to the nuclei. In three cases (3/20, 15%), focal unequivocal cytoplasmic staining was also exhibited. Strong positive nuclear staining for Fra-1 was easily seen in all types of breast carcinomas. However the nuclear/cytoplasmic concomitant immunoreactivity was observed in all types of breast carcinomas. A clear shift in Fra-1 immunoreactivity, from an exclusively nuclear to a simultaneous nuclear and cytoplasmic localization was noticed in 90.2% (37/41) of breast carcinomas. No inverse relationship between Fra-1 and ER and PR protein levels was noticed in malignant tumors. The relative expression level of Fra-1 was not correlated with the expression of ErbB2. ***Conclusion:*** The overall expression, pattern and intensity of Fra-1 proteins were correlated with breast oncogenesis. Overexpression of Fra-1, leading to a persistent high cytoplasmic accumulation, may play a role in the process of breast carcinogenesis.

Key words breast cancer; Fra-1; transcription factor; immunohistochemistry

Transcription factor activator protein 1 (AP-1) is thought to play an important role in regulating of the gene expression pattern in response to external stimuli. It is composed of transcription factors belonging to Jun and Fos families. In mammalian cells, three members of the Jun family (c-Jun, JunB, and JunD) and four members of the Fos family (c-Fos, FosB, Fra-1, Fra-2) have been identified to date [1].

All AP-1 proteins are characterized by a basic leucine-zipper region for dimerization and DNA-binding. As the transcription factors, c-Fos and FosB proteins harbor a C-terminal transactivation domain, but Fra-1 and Fra-2 lack this region. Since Fra-1 and Fra-2 were shown to inhibit c-Fos- and c-Jun-dependent transactivation in a transient-transfection assay, it has been proposed that these proteins act as negative regulators which limit the duration of the AP-1 response [2]. However, emerging evidence suggests an important role for Fra-1 in cell motility, invasion, and progression of the transformed state in several cell types [3–5]. The recent data indicated that overexpressed Fra-1 in fibroblasts causes anchorage-independent growth and oncogenic transformation [6, 7]. A high level of Fra-1 expression is found in some tumors and tumorigenic cell lines [8–11]. These results suggested that Fra-1 might be involved in malignant progression. However, few reports deal with the expression and location of Fra-1 protein in the cells of breast tumor tissues.

In this study, we conducted retrospective study using 61 paraffin-embedded breast tumor tissues to investigate Fra-1 expression at the protein level by immunohistochemistry. The correlations of Fra-1 with differentiation, estrogen receptor (ER), progesterone receptor (PR) and ErbB2 receptor status were analyzed in breast cancer patients in order to further explore the role of Fra-1 in the

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diagnosis of breast cancer.

Materials and methods

Collection of breast tissue samples

A total of 61 breast tissue specimens were collected at 307 Hospital (China) from patients undergoing surgery. All tumors tissue samples were fixed immediately after surgical removal in 10% buffered formalin and embedded in paraffin.

Clinical and pathological features

Sixty-one patients were all females,ranging from 31 to 72 yeas old (mean 47 years old). A total of 61 breast tissue specimens were composed of 20 benign breast tissues and 41 malignant breast tissues. Malignant breast tissues were constituted by 31 invasive ductal carcinomas, 4 invasive lobular carcinomas and 6 invasive ductal -lobular carcinomas. Among 41 malignant breast tissues, estrogen receptors were positive in 19 tissues and negative in 22 tissues, progesterone receptors positive in 17 tissues and negative in 24 tissues, ErbB2 receptors positive in 18 tissues and negative in 23 tissues.

Immunohistochemistry

All breast tissues were retrospectively analyzed by immunohistochemical SP three step method. Omission of the primary antibody and substitution by PBS and nonspecific immunoglobulin were used as negative controls.

Assessment of Fra-1 expression

Fra-1 expression is purely nuclear or mixed nuclear and cytoplasmic. Nuclear reactivity was scored as high (> 75% cell positive) or low (< 75% cell positive). Cytoplasmic reactivity, when present (positive) was scored as high (strong staining) and low (weak staining). The percentage of tumor cells showing nuclear or cytoplasmic Fra-1 reactivity was recorded semi-quantitatively at × 400 magnification after examining the entire histologic section.

Statistical analysis

Data were analyzed using standard statistical software SPSS version 13.0 as previously described. Fisher's Exact Test was used to determine the significance of the association of the different factors. $P < 0.05$ was considered significant.

Results

Expression of Fra-1 in benign and malignant breast tissues

The results of the immunohistochemical study of 61 breast tissues were summarized in Table 1. No staining was observed in the absence of the primary antibodies or with nonspecific immunoglobulin. Nuclear expression of Fra-1 was detected in all neoplastic tissues.

Table 1 Fra-1 expression in benign and malignant breast tissues by immunohistochemistry

Histological type	*n*	Fra-1 staining score*			
		Nuclear	*n*	Cytoplasm	*n*
Benign	20	High	11	High	0
		Low	9	Low	3
		Negative	0	Negative	17
Carcinomas	41	High	30	High	14
		Low	11	Low	23
		Negative	0	Negative	4

* Fra-1 staining score was as follows: for nuclear staining: high, > 75% positive cells and strong staining; low, < 75% positive cells and weak staining; for cytoplasmic staining: high, strong staining; low, weak staining

Expression of Fra-1 in benign breast tissues

In 85% of benign tumors (17/20), the immunoreactivity for Fra-1-recognizing antibody was exclusively restricted to the nuclei. Fig. 1 showed the nuclear immunoreactivity for Fra-1 was identified mostly in the epithelial cells. Generally, the nuclei of infiltrating inflammatory cells were not stained. Moreover, the nuclear staining was not observed in all of the epithelial cells. In most cases (85%), the immunoreactivity is present only in the nuclei. In three cases (15%), focal unequivocal cytoplasmic staining was also exhibited.

Expression of Fra-1 in malignant breast tissues

In contrast to benign breast tissues, strong positive nuclear staining for Fra-1 was evident in all types of breast carcinomas, either diffuse (100% of the tumor cells positive) or focal (> 75% of the tumor cells positive). Moreover, a variable degree of cytoplasmic staining was easily observed in 90% breast carcinomas, although the immunoreactivity in cytoplasma was weaker than in nuclear. However, cytoplasmic localization of Fra-1 was rarely observed in adjacent peritumoural tissues. In normal epidermal tissues a weak immunoreactivity was only restricted to nuclear of basal cell lalyer. In 56% of cases in breast carcinomas (23/41), strong nuclear and weak cytoplastic immunostaining was observed. It is noteworthy that in some invasive ductal carcinomas (34%, 14/41), strong nuclear and cytoplasmic double staining was very pronounced (Fig. 2).

The differential expression of Fra-1 in breast tissues

Fra-1 protein immunostaining was usually weak in normal tissues or well-differentiated adjacent peritumoural tissues and positive reactivity restricted to nuclear. Whearas in poorer differentiated region in the same section the expression of Fra-1 protein presented in nuclear

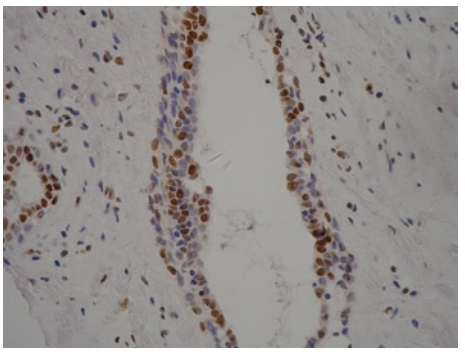

Fig. 1 The immunohistochemical staining of Fra-1 in benign breast tumors (× 400)

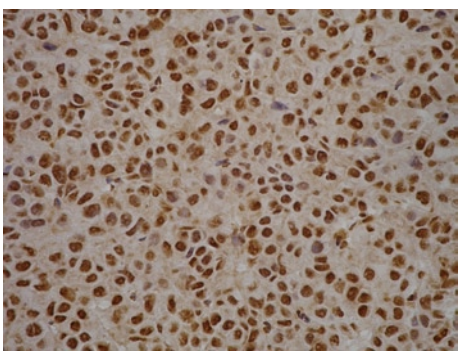

Fig. 2 The strong nuclear and cytoplasmic double staining in invasive ductal carcinomas (× 400)

and cytoplasmic simultaneously. In addition, Fra-1 expression was observed in all types of breast carcinomas.

The differential cytoplasmic expression of Fra-1 in benign and neoplastic breast tissues

The results provided in Table 2 showed an association between the cytoplasmic expression of Fra-1 and breast neoplastic tissues. Among 20 benign tissues, three cases were weak cytoplasmic staining (the positive rate 15%). Among 41 malignant tissues, 23 cases were weak cytoplasmic staining, 14 cases strong cytoplasmic staining. The positive rate was 90%. There was a significant relationship between the cytoplasmic expression of Fra-1 and breast carcinomas ($P = 0.00$).

Assessment of Fra-1, ER, PR and ErbB2 expression

No inverse relationship between Fra-1 and ER and PR protein levels was noticed in malignant tumors. The relative expression level of Fra-1 was not correlated with the expression of ErbB2 (Table 3).

Discussion

Fra-1 may play critical role in contributing to malig-

Table 2 Correlation of Fra-1 cytoplasmic expression with benign and malignancy

Tumor	*n*	Cytoplasm Fra-1 expression		*P*
		Positive (*n*)	Positive (%)	
Benign	20	3	15.0	0.000
Malignancy*	41	37	90.2	

* The positive cytoplasmic immunohistochemical reactivity of Fra-1 in breast carcinomas includes strong and weak staining

Table 3 Correlation of Fra-1 cytoplasmic expression with other prognostic factors

Characteristics	*n*	Cytoplasm Fra-1 expression		*P*
		Positive (*n*)	Positive (%)	
ER				
Negative	20	17	77.3	1.00
Positive	19	15	78.9	
PR				
Negative	24	21	87.5	0.128
Positive	17	11	35.3	
HER-2				
Negative	23	18	78.3	1.00
Positive	18	14	77.8	

nancy by altering programs of cell growth, differentiation and development. A tight association of Fra-1 expression with highly invasive breast cancer cell lines has been demonstrated. Fra-1 could induce the expression of osteopontin (OPN), thrombospondin and CD44 which are involved in metastasis in human mammary tumors [3, 4]. The effects of Fra-1 on motility and invasion were observed in four highly invasive and nine weakly invasive human breast cancer cell lines by cDNA array technology. Among 24 differentially expressed genes, Fra-1 expression levels was significantly enhanced in the highly invasive cells. Similar results were found by cDNA array technology in 22 human mammary epithelial cell strains/lines (breast cancer cell lines and cells derived from primary or metastatic breast cancers and reduction mammoplasties) [2]. It has been also reported that Fra-1 correlated with the absence of ER expression and a more undifferentiated phenotype in breast cancers [12]. Furthermore, Fra-1 was efficient for stimulating MMP-9, MMP-1, VEGF and cyclin D1 production in MCF7 cells [13]. Obviously, Fra-1 may influence cell proliferation, invasion and angiogenesis as well. Therefore, some authors suggest that Fra-1 might be a valuable diagnostic marker in breast cancer.

To further investigate the significance of Fra-1 in the diagnosis of breast cancer, the expression of Fra-1 was investigated by immunohistochemistry with the anti-Fra-1 antibody in neoplastic breast diseases ranging from benign fibroadenoma to very aggressive undifferentiated carcinoma. The correlations of Fra-1 expression with other indicators of breast carcinoma prognosis (ER, PR and ErbB2 receptors) was analyzed. In the study reported

here, all neoplastic breast tissues, either benign or malignant breast tissues, were nuclear immunoreactive for Fra-1-recognizing antibody, whereas tumor adjacent normal tissue showed a much weaker nuclear immunoreactivity only restricted to some epithelial cells. The patterns of Fra-1 expression by benign neoplastic cells were predominantly nuclear. In only 3 of 20 cases of fibroadenomas or hyperplastic adenosis/ fibroadenomas, nuclear expression coexisted with weak cytoplasmic reactivity. However, the nuclear/cytoplasmic concomitant immunoreactivity was observed in all types of breast carcinomas. A clear shift in Fra-1 immunoreactivity, from an exclusively nuclear to a simultaneous nuclear and cytoplasmic localization was noticed in 90.2% of breast carcinomas. Moreover, we cannot exclude a slight increase in Fra-1 expression not detectable by immunohistochemistry. Noteworthy, Fra-1 staining differed among malignant neoplastic tissues and tended to be correlated with the differentiation of breast carciomas. The data indicate that the expression pattern and intensity of Fra-1 proteins were correlated with epithelial cell oncogenesis.

Fra-1 lacks a transcriptional transactivation domain and it is thought, in partnership with c-Jun, to drive the expression of genes [2]. Its overexpression results in the transcription of progression-associated genes and induction of epithelial mesenchymal transition [14]. The recent evidence have also indicated a potential role for Fra-1 in abnormal differentiation and transformation of lung cells [15]. However, the role played by Fra-1 in malignancy remain unclear. It is known that many transcription factors shuttle between the nucleus and the cytoplasm. The synthesis of Fra-1 is supposed to start in the cytoplasm. However when it was overexpressed, nuclear import might be hampered. Saturation of a nuclear import mechanism, leading to a persistent high cytoplasmic concentration of the proteins, may also play a role in the process of breast carcinogenesis.

In conclusion, our data by immunohistochemical analysis of breast tissues, including benign and malignant breast carcinomas, reveal that Fra-1 protein levels in the cytoplasm may be an indicator for the diagnosis of human breast cancer. These data may highlight the significance of therapy based on the blockage Fra-1 functions of breast cancers.

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The clinical research of elemene emulsion combined with FOLFOX4 regimen in the treatment of advanced gastric carcinoma

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Abstract ***Objective:*** The aim of this study was to observe the effects and adverse reactions of elemene emulsion added to the chemotherapy in the treatment of advanced gastric carcinoma (AGC). ***Methods:*** Forty-nine patients were divided randomly into two groups, elemene emulsion group (25 cases, treated with chemotherapy and elemene emulsion) and chemotherapy group (24 cases, treated with chemotherapy only). All patients received chemotherapy. The clinical effects and adverse reactions were evaluated after four cycles. ***Results:*** The response rate (RR) were 60% in elemene emulsion group and 41.7% in chemotherapy group respectively ($P < 0.05$). The median time to progression and overall survival in elemene emulsion group and in chemotherapy group were 7.1 months and 11.0 months vs 5.2 months and 9.3 months ($P < 0.05$). A lower rate of neutropenia, nausea, vomiting and diarrhea occurred in elemene emulsion group compared with chemotherapy group ($P < 0.05$), and there was significant difference in the elevation of life quality as well (48% vs 25%; $P < 0.05$). ***Conclusion:*** Elemene emulsion in combination with FOLFOX4 regimen can improve the efficacy, decrease the incidence of side effects of chemotherapy and elevate the life quality and prolong the survival time in AGC.

Key words elemene emulsion; advanced gastric carcinoma (AGC); FOLFOX4 regimen

Gastric cancer is one of the most common cancer in China. Owing to poorly early diagnosis, most patients were usually diagnosed at a relatively advanced stage with metastasis to other organs. Various chemotherapy regimens have been developed for advanced gastric cancer (AGC) patients based on the understanding that chemotherapy can inrease the length and quality of life (QOL) compared with best supportive care (BSC). While with poor performance status and inadequate immunological function, most patients still have a miserable outcome. Therefore, some reporters tried to plus a traditional Chinese medicine to chemotherapy in order to increase the response rate (RR) and enhance the QOL [1]. Elemene, a compound isolated from Curcuma wenyujin, has been proven to elevate the immune function and suppressed the growth of tumor cells with no cytotoxicity. This study was disigned to observe the clinical activity and safety of elemene emulsion combined with FOLFOX4 for the treatment of AGC.

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Materials and methods

Patients

The inclusion criteria for AGC patients were: (1) pathologically proved locally advanced (non-resectable) or metastatic gastric cancer; (2) age more than 18 years; (3) measurable lesion that can be accurately measured in at least one dimension; (4) Karnofsky performance status (KPS) ≥ 70; (5) adequate bone marrow functions; (6) adequate hepatic function and renal function; (7) estimated life expectancy of at least three months and no other malignancies.

The exclusion criteria for patients included: (1) preexisting peripheral toxicity ≥ grade 2 of the National Cancer Institute Common Toxicity Criteria (NCI-CTC); (2) concurrent or prior malignancy; (3) central nervous system metastases; (4) active infection; (5) other uncontrolled underlying medical conditions that would impair the ability of the patients to receive the planned treatment; (6) concurrent treatment that interfered with the study evaluation.

All patients provided written informed consent before treatment that contained information on chemotherapy drug, treatment schedule, and toxicity.

Treatment methods

Patients were randomly divided into chemotherapy group and elemene emulsion group. All patients received the modified FOLFOX4 regimen: oxaliplatin 85 mg/m^2 as a 2 h infusion on d1, and leucovorin 50 mg as a 2 h infusion followed by bolus 5-fluorouracil (FU) 400 mg/m^2 and a 22 h infusion of 5-FU 600 mg/m^2 on d1 and d2. This treatment was repeated every 2 weeks. All patients received anti-emetic therapy prior to chemotherapy.

In the elemene emulsion group, chemotherapy regimen was the same, and elemene emulsion (DaLian Holley KingKong Pharmaceutical Co. Ltd., China) 500 mg /d (d1–14) was used intravenously.

Treatment was continued until disease progression or unacceptable toxicity occurred or the patient declined further treatment.

Evaluation of RR and toxicity

Physical examination, complete blood counts, biochemistry tests, and assessment of symptoms and signs were carried out for the patients within 3 d before enrollment and every week during the study period. CT scans or MRI were carried out every 4 cycles of chemotherapy. According to the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines [2], responses included complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). The RR was defined as the sum of CR and PR rates. The time to progression (TTP) was calculated from the date of the beginning of treatment to that of clinical progression. The overall survival (OS) was defined as the period from the date of treatment to the death of patients or the last follow-up appointment. Toxic effects were evaluated according to the WHO standard.

Evaluation of the QOL

KPS was served to evaluate the QOL, KPS score increased ≥ 10 was considered as improvement, the score decreased ≥10 was considered as progression, the score increased or decreased < 10 was considered as stable.

Statistical analysis

All the analyses were performed using SPSS software (Version 11.0). Data were processed by Chi-square test and rank sum test, P value < 0.05 was defined statistical significance.

Results

Patients characteristics

Forty-nine patients were enrolled in this study from August 2006, to October 2009. 24 patients in the chemotherapy group with the median age 53 years (range: 32 to 74 years) included 14 males and 10 females. 9 cases were moderately differentiated adenocarcinoma, 8 cases poorly differentiated adenocarcinoma, 5 cases mucinous carcinoma, 2 cases signet ring cell carcinoma. 7 cases and 17 cases were classified as stage IIIB and stage IV, respectively. In the elemene emulsion group, 25 patients with the median age 52 years (range: 31 to 75 years) included 14 males and 11 females. 8 cases were moderately differentiated adenocarcinoma, 8 cases poorly differentiated adenocarcinoma, 6 cases mucinous carcinoma, 3 cases signet ring cell carcinoma. 9 cases and 16 cases were classified as stage IIIB and stage IV, respectively. Most patients had one or more involved regions, the main metastatic sites were the abdominal lymph nodes, the liver and lung, with 13/9/4 cases and 12/11/6 cases in the chemotherapy group and elemene emulsion group, respectively. No significant difference was observed in any clinical characteristics between the two groups (P > 0.05).

Response

All the patients were evaluated for response and no patient was excluded from the efficacy analysis because of severe side effects.

The median therapy cycles were 9.2 ± 3.7 and 9.6±3.9 in the elemene emulsion group and chemotherapy group (P < 0.05). Of the patients in elemene emulsion group, 3 cases achieved a CR and 12 PR, 5 had SD, and 5 PD, with an RR of 60.0%. Of the patients in chemotherapy group, 2 cases achieved a CR and 8 PR, and 9 had SD and 5 PD, with an RR of 41.7%. The difference of RR between two groups was significant (P < 0.05).

The median follow-up duration was 18 months. The median TTP and OS in the elemene emulsion group was 7.1 months and 11.0 months, respectively. The median TTP and OS in the chemotherapy group was 5.2 months and 9.3 months, respectively, and significant difference was found between the two groups (P < 0.05).

Comparison of adverse events

The main adverse events included hematologic toxicities, gastrointestinal reactions and neuropathy. The frequencies of neutropenia, nausea and vomiting, and diarrhea were lower in the elemene emulsion group compared with those in the chemotherapy group (P < 0.05). Neuropathy of different grades were observed with no significant difference in two groups (P > 0.05). Hepatic or renal toxicities and oral mucosa ulcer were relatively infrequent and slight. The common toxicities were listed in Table 1.

QOL

In the elemene emulsion group, 12 patients achieved KPS score improvement and 6 cases stable, while in the chemotherapy group, only 6 patients achieved improvement and 3 stable, the enhancement rates of KPS were

Table 1 Comparison of toxicities between two groups

Toxicity	Elemene emulsion group (n = 25)				%	Chemotherapy group (n = 24)				%
	1	2	3	4		1	2	3	4	
Neutropenia	2	4	1	1	32.0	6	4	4	3	70.8*
Neurosensory	2	2	0	0	16.0	4	2	0	0	25.0
Nausea/vomiting	4	5	0	0	36.0	5	8	0	0	54.2*
Diarrhea	2	3	0	0	20.0	6	3	0	0	37.5*

* $P < 0.05$

48% vs 25% in two groups with significant difference ($P < 0.05$).

Discussion

Elemene is a mixture isolated from more than 50 Chinese herbs and plants, such as Curcuma wenyujin [3]. Elemene is mainly composed of β- and δ- and γ-gelemene, with β-elemene accounting for 60%–72% of all three isoforms. β-Elemene can inhibit the growth of different tumor cells, induce apoptosis, anti-angiogenesis, anti-tumor metastasis, also shows synergistic effects in combination with other chemotherapeutic drugs *in vitro* and *in vivo* experiments [4–8]. β-elemene exerts anti-cancer potential in brain [9], lung [10], malignant pleural effusion [11], gastric carcinomas [12].

Although the efficacy of palliative chemotherapy is now widely accepted and many clinical trials containing new cytotoxic agents (such as docetaxel, irinotecan, oxaliplatin, capecitabine, *et al*) have been conducted, no chemotherapeutic regimen has been accepted as the consensus standard treatment for AGC.

Oxaliplatin, a third generation platinum compound, is a new-generation alkylating agent that inhibits DNA replication, it appears to have a better safety profile than cisplatin, such as nausea, vomiting, neurotoxicity. The neuropathy of oxaliplatin primarily manifests as cumulative, reversible sensitivity to cold. Oxaliplatin has shown efficacy against many tumor cells, in addition, it has demonstrated additive or synergistic activity when combined with other drugs. Oxaliplatin is typically administered in combination with 5-FU and leucovorin known as FOLFOX for the treatment of colorectal cancer. FOLFOX4 regimen shows a manageable toxicity profile and the RR of AGC patients to this regimen is 42.5%–55.2%, the median TTP and OS were 5.9 months and 10.4 months, respectively, and FOLFOX4 regemen is now recommended as one of the most commonly used for fisrt-line and salvage treatment of AGC [13, 14].

Elemene emulsion combined with FOLFOX4 was proven to produce a better QOL and to increase the effects of chemotherapy. A previous study suggested that elemene emulsion combined with OLF regimen might have a more beneficial effect on RR (55.9%) [12]. In our study, the RR was 60%, the median TTP and OS was 7.1 months and 11.0 months, respectively, which is comparable with above-mentioned report. Surprisingly, when elemene emulsion was used, the RR was even higher than that in the ToGA trial [15], it might be explained by the relatively inadequate patients enrolled in our study, and this needs further investigation.

To date, the assessment of QOL is also important and some trials have reported the improvement of QOL from chemotherapy. In our study, compared with FOLFOX4 regimen alone, the addition of elemene emulsion could elevate the RR, prolong the TTP and OS, also improve the KPS status of patients, which means elemene emulsion has a synergistic activity with chemotherapy. Moreover, the lower rate of toxicity, such as neutropenia, nausea and vomitting, diarrhea was demonstrated in the elemene group, which also indicates elemene emulsion can decrease the adverse effects of FOLFOX4 regimen.

Above all, elemene emulsion, in combination with FOLFOX4 regimen, can increase the antitumor effects and decrease the adverse effects of chemotherapy, which needs further clinical research.

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The effect of pre-low-dose X-ray radiation on tumor inhibition of HepG2 cells in tumor-bearing nude mice

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Abstract ***Objective:*** The aim of this study was to discuss the effect of pre-low-dose X-ray radiation on P53, Bcl-2 and apoptosis of HepG2 cells in tumor-bearing nude mouse, and further explore the mechanism of low doses radiation. ***Methods:*** HepG2 cells were implanted subcutaneously into nude mice. 14 days after the implanting, these mice were divided into 6 groups randomly, S group (sham-irradiation 0 cGy), D1 group (7.5 cGy, dosage rate = 7.5 cGy/min), D2 group, (200 cGy, dosage rate = 100 cGy/min), D1 + 2 h + D2 group, D1 + 6 h + D2 group and D1 + 12 h + D2 group. Tumor-bearing mice in each experimental group were executed at 24 h after the last irradiation. P53 and Bcl-2 were detected by immunohistochemical staining, the tumor tissues apoptosis were detected in site (Tunel). ***Results:*** Each combined exposure groups (D1 + 2 h + D2 group, D1 + 6 h + D2 group and D1 + 12 h + D2 group) compared with the D2 group, the percentages of positive P53 and Bcl-2 were decreased obviously, and the apoptotic indexs were increased ($P < 0.01$). ***Conclusion:*** Pre-low-dose radiation combined with the conventional radiation can increase the apoptosis of tumor tissues by decreasing the expression of P53 and Bcl-2, it can enhance the anti-tumor effect of conventional radiation, and it can have actual clinical significance on supporting radiotherapy.

Key words low-dose radiation; HepG2; apoptosis; apoptosis-related proteins; Tunel

Apoptosis is the main form of cell death due to the anti-tumor effect of ionizing radiation, it relates to a series of tightly regulated process, which regulated by multiple genes [1, 2]. Though its specific procedure has not been entirely clear, some relatively conservative genes, such as tumor suppressor P53 gene and Bcl-2 gene family and a few others, have been recognized by the public through the development of molecular bio-technology [1, 3]. By enhancing body's immune system, low-dose radiation (LDR) can induce stimulation of immunity to produce a wide range of adaptive responses, and currently it is the widely accepted theoretical foundation of anti-tumor effect of LDR [4, 5]. By testing on nude mice which lack of immune system, does LDR indicate whether it increases the post-exposure of conventional radiotherapy efficiency on tumors? In this experiment we used nude mice, which were implanted subcutaneously by HepG2 cells as experimental subjects, to study the effect of LDR before conventional radiotherapy on the expression of P53, Bcl-2 and apoptosis. And further investigate the molecular mechanism of this procedure in order to provide new theoretical support of the clinical application of low-dose radiation.

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Materials and methods

Source of cell line

HepG2 cell was provided by the Central Laboratory of The Affiliated Hospital of Medical College Qingdao University; experimental mice: nude mice (purchased from Qingdao Institute for Drug Control, China), male, weight 20 to 22 g, 3 to 4 weeks old, conventional breeding. License No. SCXK (Lu) 20030010, raised in specific pathogen free (SPF) conditions, laminar flow clean bio-frame.

Exposure conditions

Nude mice were put into a 15 cm × 15 cm × 35 cm wooden box; Varis 23EX accelerator; exlipse radiation treatment planning system; D1 exposure conditions: radiation field was 15 cm × 15 cm, source skin distance was 100 cm, lead weight and 0.5 cm tissue compensator were put between the sourse and the box. By dose verification dose rate = 7.5 cGy/min (equipment dose rate = 100 cGy/min), 1 min exposure time. D2 exposure conditions: radiation field was 15 cm × 15 cm, source skin distance was 100 cm, 0.5 cm tissue compensator was put between the sourse and the box. By dose verification dose rate =100 cGy/min, 1 min exposure time.

Table 1 After irradiation the changes of tumor weight, P53, Bcl-2 expression rate and apoptotic index (AI) in tumor tissue

Group	Tumor weight (g)	P53 expression rate (%)	Bcl-2 expression rate (%)	AI (%)
S	1.21 ± 0.19	41.13 ± 6.83	69.31 ± 6.13	9.73 ± 4.56
D1	1.20 ± 0.17	37.79 ± 6.68	63.62 ± 5.89	11.96 ± 5.84
D2	1.18 ± 0.21	24.51 ± 7.12	34.54 ± 6.62	27.37 ± 4.41
D1 + 2 h + D2	1.18 ± 0.18	22.36 ± 6.70	28.49 ± 7.05	33.13 ± 5.79
D1 + 6 h + D2	1.14 ± 0.19	21.72 ± 6.49	24.08 ± 7.21	39.69 ± 6.13
D1 + 12 h + D2	1.15 ± 0.20	21.89 ± 6.79	23.59 ± 7.28	38.34 ± 5.03

Establishing xenografts in nude mice and collecting specimen

HepG2 cells were cultured in 1640 medium with 10% fetal bovine serum. When HepG2 cells were in logarithmic growth phase and cell viability was counted greater than 95% by trypan blue staining, adjusted the cell concentration to 6×10^6/L. Under sterile conditions, 0.2 mL of single tumor cells suspension without serum was implanted subcutaneously at the left groin of nude mice, the whole process was completed within 1 h. 3 in all 65 nude mice appeared self-limiting, tumor nodules were disappeared at d7, d9 and d12 respectively, and another mouse didn't even have tumor nodule. Tumor formation rate was 93.85%. 14 days after implantation, tumor nodules grew into 0.6 cm to 1.0 cm in diameter. The tumor-bearing node mice were randomly divided into 6 groups (10 mice in each group). Sham-irradiation (S) group was exposed 0 cGy; D1 group was exposured 7.5 cGy; D2 group was exposed 200 cGy; D1 + 2 h + D2 group was exposed D2 dosage 2 h after D1 dosage; D1 + 6 h + D2 group was exposed D2 dosage 6 h after D1 dosage; D1 + 12 h + D2 group was exposed D2 dosage 12 h after D1 dosage. All node mice in every group were executed 24 h after the last exposure. All subcutaneous tumor nodules were dissected completely, generally observed the tumor specimens with unaided eyes, then weighed in the balance with accuracy of 0.01 g. After that fixed the tumor specimens in 10% formalin immediately, then embedded them in paraffin. Specimens were sliced with the thickness of 6 μm.

Detection items

(1) PowerVision™ two steps method immunohistochemical staining was used to detect the expression of P53 and Bcl-2. Replace Primary antibody by PBS as negative control, the known positive biopsy as a positive control. Randomly prepared each sample into 5 slices, each of which was observed 10 high-power fields (400 X) under microscope to count the expression rates of P53 and Bcl-2 (expression rate=positive cells / all counting cell × 100%). (2) The Tunel detection in situ was used to detect apoptosis, randomly prepared each sample into 5 slices, each of which was also observed 10 high-power fields (400 X) under fluorescence microscope with red background to count the apoptotic cells with green fluorescent staining (fluorescein 12-dUTP). Apoptotic index (AI) = apoptotic cells / (apoptotic cells + normal cells).

Statistical analysis

All statistical data were analyzed using SPSS (version 11.5). Tumor weight, the expression rates of P53, Bcl-2 and AI were demonstrated in the form of $\chi \pm s$ and analyzed with t test.

Results (Table 1)

The results of expression of P53

Compared to S group, all samples exposed to radiation showed lower percentages of positive for P53. In each paired comparison, D1 group showed no distinctive statistic difference ($P > 0.1$), yet D2 group proved there was a statistical significance ($P < 0.05$), and other groups all displayed lower percentages of positive for P53 in their comparisons ($P < 0.005$).

The results of expression of Bcl-2

Similar to previous test, compared to S group, all samples exposed to radiation experienced lower percentages of positive for Bcl-2. Even D1 group has a statistical distinction ($0.01 < P < 0.05$), all other groups has lower numbers to prove their statistical significance ($P < 0.005$). And all the combined exposure groups compared with the D2 group Bcl-2 also showed a clear decline in the percentage ($P < 0.05$).

The results of Tunel apoptosis detection

In comparison with S group, D1 group showed no statistical significance ($P > 0.1$), D1 + 2 h + D2 group had a higher apoptotic index in contrast to D2 ($0.01 < P < 0.05$) , Both of D1 + 6 h + D2 group and D1 + 12 h + D2 group had higher apoptotic index comparing to D2, that each of both had P-value smaller than 0.005.

Discussion

In recent domestic and foreign experimental studies and clinical observations, mammals including humans, who were exposed to low-dose radiation, can induce comprehensive adaptive responses, in the performance of epigenetic changes in cells and changes in immune func-

tion [6, 7]. Low-dose irradiation can generate damages on chromosomes that would lead to apoptosis. Its mechanism correlates to the cell signal transduction and the activities of antioxidant system. Moreover, on genetic regulation, that LDR can influence certain genetic expression leads to related variances in protein synthesis. The effect of radiation on gene regulation has been the primary focus of researchers worldwide. However, most researchers believe that the dependence on LDR is not sufficient in treatment of tumors [8], but by combining with other treatment of tumors, specifically with conventional radiation, LDR can enhance the inhibition [9, 10]. In this experiment, simply through low dose irradiation of 7.5 cGy, we can only increase tumor apoptotic index by three percents, which is hardly enough in treatment of the repulsive tumor cells that are growing rapidly in numbers. Nonetheless, with subsequent conventional radiation, LDR can significantly improve the tumor apoptotic index. And compared with pure conventional irradiation, pre-LDR can play a synergistic role with subsequent conventional irradiation, also significantly increased killing effect on tumor cells.

P53 [11] protein plays an important role in cell cycle as one of the core factors. As for now, P53 gene is believed as the highest of human tumor-associated genes. p53 is a tumor suppressor gene, it locates on human chromosome 17p13.1, involved in cell cycle regulation, DNA repair, apoptosis and other important biological functions. P53 gene could be either wild-type or mutant, and its product also has these two distinctions. Wt-P53 protein has a broad-spectrum inhibition of tumor, but its structure is very unstable, half-life only lasts for few minutes, it is hard to detect by immunohistochemistry. If p53 gene occurred mutation, its tumor inhibition is reduced, might lead to tumor. The mutant P53 protein in comparison, is more consistent and have longer half-life for detection. This study found that each group of tumor tissues in nude mice bearing HepG2 cells showed various levels of p53 protein expression in cells. After exposed to 7.5 cGy irradiation, the percentage of P53 expression rate only showed a minimal decrease compared with sham-irradiation ($P > 0.1$). Yet exposed to 200 cGy irradiation, whether it has been exposed after the LDR, the drop in percentages of P53 expression rate is more obvious. Moreover, drop is much more significant in the groups which has been exposed pre-LDR ($P < 0.005$).

The current studies confirm that Bcl-2 protein locate in mitochondria, endoplasmic reticulum and nuclear membrane. As an oncogene, its physiological function is to suppress apoptosis and prolong cell life. By preventing the release of mitochondrial cytochrome C and gathering glutathione in nucleus, Bcl-2 protein affect the redox state of cells, and thus play a role in anti-apoptotic [12]. Currently reports on the effects of HepG2-bearing nude mice exposed to low doses of radiation in the molecular level have been rare. In this study, various levels of Bcl-2 protein expression were found in tumor tissues of HepG2-bearing nude mice in every group. After exposed to 7.5 cGy irradiation, the percentage of Bcl-2 expression rate showed apparent decrease compared with sham-irradiation ($0.01 < P < 0.05$). Moreover, exposed to 200 cGy irradiation, whether it has been exposed after the LDR, the drop in percentages of Bcl-2 expression rate is more significant ($P < 0.005$). And compared with only exposed to 200 cGy, the combined exposure groups also showed a significant drop in percentages of Bcl-2 expression ($P < 0.05$).

Wt-P53 can together up-regulating the expression of Bax and reducing expression of Bcl-2 to complete the promotion of apoptosis. According to the statistics in this study, mutant P53 protein expression decrease as Bcl-2 protein expression decrease, representing a correlation between these two. Meanwhile the study also indicates as the elapsed time between the low-dosage and conventional irradiation gets extended, apoptotic protein Bcl-2's expression rate decreased gradually. Such variation in gene regulation was showed in the form of apoptotic index increasing. Two h after the pre-LDR provide conventional irradiation, we fail to obtain a significant increase in apoptotic index ($0.01 < P < 0.05$). If we increase the time interval to 6 h instead of 2, the apoptotic index could reach to a significant increase. And continuing such procedure to reach the time to 12 h, the index will drop moderately. There is evidence that the effect on tumor cells is connected to the length of the elapsed time between the two treatments of LDR and conventional. Xia [13] and his team made HepG2 cells *in vitro* for object of study, analyzed the impact of pre-γ ray LDR (5.0 cGy) following by high-dose irradiation in cell cycle arrest, by using flow cytometry. The results showed that HepG2 tumor cells *in vitro* exposed by high-dose irradiation 4 h after pre-LDR accumulated in G2/M phase, and high-dose irradiation 8 h after pre-LDR could promote HepG2 tumor cells through G2/M phase. As we all know, low-LET radiation is most destructive on G2/M phase cells, therefore it can be concluted that the appropriate time between the pre-LDR and conventional radiotherapy should be between 4 to 6 h.

This study proves the treatment of low-dose irradiation before conventional irradiation can decrease the expression of P53 and Bcl-2, and it can significantly increase the destruction of tumor cells. Furthermore, the effect of this operation is highly correlated with the elapsed time between two irradiations. Provide a new theoretical basis to carry out the clinical pre-LDR combined with conventional radiotherapy.

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Evaluation of the optimal field arrangement for conformal radiotherapy for prostate cancer patients

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Abstract ***Objective:*** The aim of this study was to evaluate the optimal field arrangement for conformal radiotherapy (CFRT) for prostate cancer patients. ***Methods:*** Thirty patients with prostate cancer of different grades and stages were treated with 3D conformal radiotherapy to minimize the dose to bladder, rectum and head of both femora using four fields (4F), five fields (5F), six fields (6F) and ARC techniques to minimize the risk of over dose to bladder, rectum and femoral heads. Patients received a total dose between 76 to 78 Gy given in 38 to 39 fractions over 7.5 to 8 weeks. ***Results:*** It was observed that V95, D95, D50 and D5 values for planning target volume (PTV) were comparatively higher when planned by 5 fields technique than when planned by fixed field technique (91%, 91%, 90% and 91.4% for skip-scan technique versus 85%, 87%, 86% and 88% by fixed field). The organs like rectum and urinary bladder get much higher dose when treated by fixed field techniques than rotation or 5 fields technique, when comparison was made for V95, V50 and DM values for rectum and urinary bladder obtained by 5 fields technique planning and 4/6 field planning, the value for 5 fields technique was found to be lower than 4/6 field technique (1%, 70% and 51% versus 13%, 91% and 55% for rectum and 4%, 25% and 51% versus 16%, 38% and 56% for urinary bladder respectively). ***Conclusion:*** Similarly for femoral heads, planning by full rotational technique had been observed to be beneficial as compared to when planning was done by fixed field technique (0%, 0% and 29% versus 0%, 1% and 28%).

Key words prostate cancer; conformal radiotherapy (CFRT); evaluation; field arrangement

Radical radiotherapy is commonly used for the curative treatment of localized prostate cancer. Dearnaley *et al* [1] recently reported that conformal radiotherapy (CFRT) techniques in the radical treatment of prostate cancer provided significant reduction of late rectal morbidity compared with conventional open field techniques.

Using prescribed doses of up to 64 Gy, rectal complications ¢grade 2, measured on the Radiation Therapy Oncology Group (RTOG) scale, were reduced from 15% to less than 5% ($P = 0.01$). This provided the foundation for the randomized Medical Research Council (MRC) RT-01 trial of dose escalation for localized prostate cancer.

The normal tissue complication probability model using the Lyman–Kutcher–Burman scheme [2–4] predicts a significant increase in rectal dose complications if the planning target volume (PTV) or treatment margins are not modified when the dose is escalated from 64 Gy to 74 Gy [5–7]. The MRC RT-01 trial protocol has anticipated these issues by specifying a two-phase approach in which the clinical target volume (CTV) is reduced for the boost phase to a volume that covers the prostate gland only, with no PTV margin. In previous studies [5–7], suitable plans were evaluated for the first phase of prostate CFRT to 64 Gy using planning margins of 10 mm, but we have not specifically addressed the situation where the PTV is equal to a prostate-only CTV.

In this study we are primarily interested in selection of the optimal beam arrangement for delivery of radiation therapy by a dose of 76 Gy to the prostate.

We had therefore evaluated a series of four fields (4F), five fields (5F), six fields (6F) and ARC techniques plans for use in the treatment delivery of dose escalation in prostate CFRT. These selected plans were compared with a series of reference plans, some of which were recommended in the MRC RT-01 trial protocol. The aim was to determine which of the 4F, 5F, 6F and ARC plans arrangements provide the best rectal sparing, with acceptable bladder and femoral head doses, for this situation.

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Patients and methods

Patients

From February 2008 till October 2010, 30 patients with histologically confirmed prostate cancer were retrospectively studied. All patients underwent staging investigations that revealed no clinical evidence of regional nodal disease or distant metastasis. The local tumour stage [8] ranged from T1c to T3c. The median age of these patients was 65 years (range 53–76 years).

For prostate CFRT, all patients were scanned using CT in a supine position with a "comfortably full" bladder. CT scan slices of 5 mm thickness were taken at 5 mm intervals from 10 mm inferior to the level of the ischial tuberosities to the apex of the bladder or to the bottom of the sacroiliac joint, whichever was greater. The CT images were then automatically transferred to a TARGET-2 planning workstation, where the CTV and relevant organs-at-risk (OARs) were outlined.

The CTV included the prostate. The OAR included urinary bladder, rectum and head of both of femora. The PTV was contoured by manual expansion of the CTV with a margin of 0.5–1 cm in X, Y and Z axis except at prostate-rectal wall interface where a margin of 0.5 was used.

Beam arrangement

The dose delivered to the prostate was 76–78 Gy. All beam arrangements were visualized using beam's eye view (BEV) display. MLC beam shaping was used to create beam apertures. Asymmetric collimation and wedges were used when necessary. X-ray beam energy used was 6 MV or 15 MV when we needed. Fig. 1 showed that all were coplanar beams. Four fields technique was by arrange (0°, 90°, 180°, and 270°) and (45°, 90°, 270°, and 315°), 5 fields by arrange (0°, 295°, 109°, 69°, and 250°). Six fields have a different arrange techniques (40°, 90°, 115°, 245°, 270°, 320°, 55°, 90°, 125°, 235°, 270°, 305°, 50°, 90°, 130°, 230°, 270°, and 310°).

Dosimetry and planning

Dose distribution was calculated with a pencil beam algorithm with pixel-by-pixel in homogeneity correction. The prescribed dose was normalized to 100% at the is centre and 95% isodose surface covered the PTV. The maximum isodose within the PTV average dose ≈ 104%. Multiple beam plans were created and compared using dose volume histograms and images before selecting the optimal plan. Digital reconstructed radiograph (DRR) (Fig. 2) image from the axial CT images was created to visualize the fields of the selected plan. The MLC field shape in linear accelerator was checked with BEV printout of all fields from the 3D planning system. Following field markings, the 3D beam plan was transferred from planning to linear accelerator computer workstation. 3D-CRT was delivered in linear accelerator with patient immobilized in supine position, field verification (Fig. 3) with online EPID was done before the first fraction and repeated every five fractions during the course of treatment. Fig. 4 showed a summary for the planning and verification steps done for the patients.

Results

Mean statistics for each of the 4F, 5F, 6F and ARC plans for CTV, PTV and organs at risk were assessed in the following tables (Tables 1–3).

Table 1 showed the dose volume histogram (DVH) analysis data for CTV, V95, D95, D50 and D5 values for CTV planned by 5F technique were 91%, 93%, 92% and 94% respectively, when the patients were planned by fixed field these above mentioned values for CTV were found to be 85%, 91%, 90% and 92% respectively.

Table 2 showed the DVH analysis data for PTV. It was observed that V95, D95, D50 and D5 values for PTV were comparatively higher when planned by 5F technique than when planned by fixed field technique (91%, 91%, 90% and 91.4% for skip-scan technique versus 85%, 87%, 86% and 88% by fixed field).

Table 3 as well as Fig. 5 and 6 showed DVH analysis for organs at risk/normal tissues, from the table it was clearly observed that organs like rectum and urinary bladder got much higher dose when treated by fixed field techniques than rotation or 5F technique. When comparison was made for V95, V50 and DM values for rectum and urinary bladder obtained by 5F technique planning and 4/6 field planning, the value for 5F technique was found to be lower than 4/6 field technique (1%, 70% and 51% versus 13%, 91% and 55% for rectum and 4%, 25% and 51% versus 16%. 38% and 56% for urinary bladder respectively). Similarly for femoral heads (Fig. 7), planning by full rotational technique had been observed to be beneficial as compared to when planning was done by fixed field technique (0%, 0% and 29% versus 0%, 1% and 28%).

Discussion

This study was carried on 30 patients with cancer prostate treated by conformal radiotherapy at the International Military Center (IMC) in the years 2008 till 2010. The aim of the study was to compare the different fields techniques used in the 3D conformal treatment for prostate cancer.

In terms of rectal and bladder sparing, it was clearly observed that organs like rectum and urinary bladder got much higher dose when treated by fixed field techniques than rotation or 5F technique. When comparison was made for V95, V50 and DM values for rectum and uri-

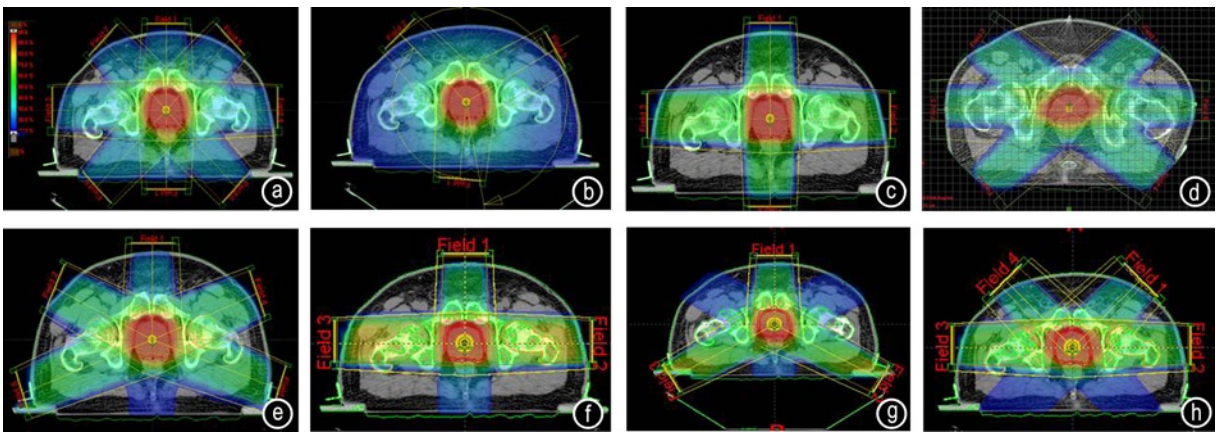

Fig. 1 Display for different fields arrangements. (a) Eight fields arrange; (b) ARC therapy; (c) Four fields technique; (d) Six fields technique; (e) Five fields arrangement; (f) Standard three fields arrangement; (g) Non standard three fields arrangement; (h) Non standard four fields arrangement

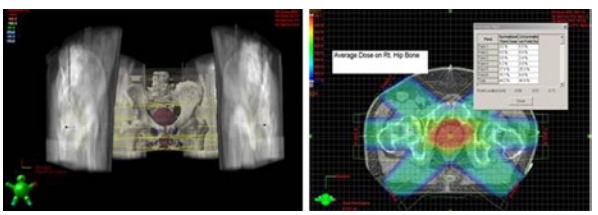

Fig. 2 DRR for six fields technique and average dose for Rt. hip bone

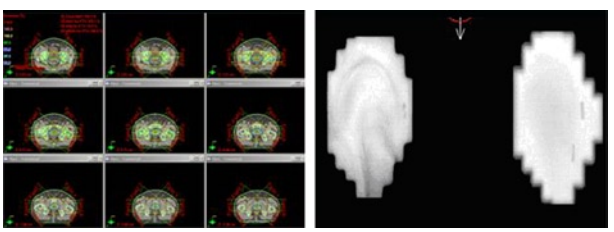

Fig. 3 Evaluation of plan portal image for anterior and lat fields

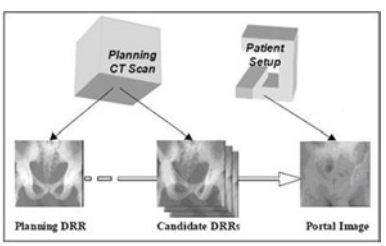

Fig. 4 The verification steps

nary bladder obtained by 5F technique planning and 4/6 field planning, the value for 5 fields technique was found to be lower than 4/6 field technique (1%, 70% and 51% versus 13%, 91% and 55% for rectum and 4%, 25% and 51% versus 16%. 38% and 56% for urinary bladder respectively). Similarly for femoral heads, planning by full rotational technique had been observed to be beneficial

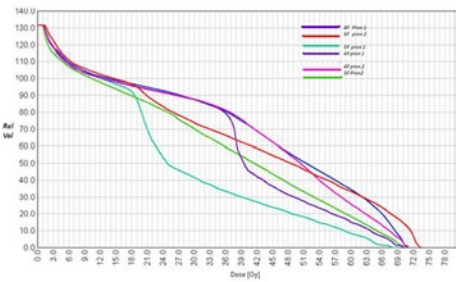

Fig. 5 Dose volume histogram displayed the bladder averages from comparing the five techniques

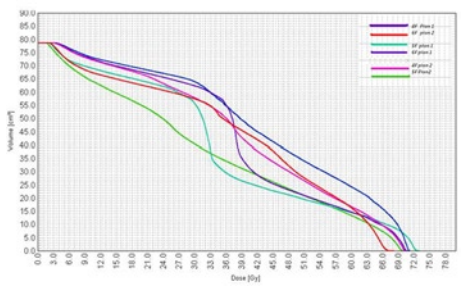

Fig. 6 This dose volume histogram displayed the rectum averages from comparing the five techniques

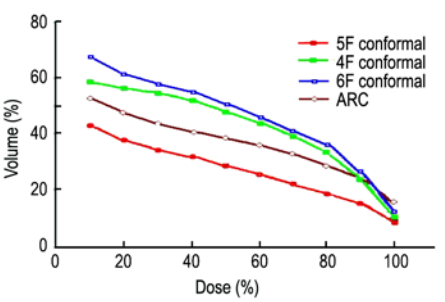

Fig. 7 This dose volume histogram displayed the femoral head averages

Table 1 Dose volume histogram analysis for CTV (%)

Technique	V95		D95		D50		D5	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
4F	85	73–95	91	86–95	90	86–93	92	88–95
6F	85	74–90	91	86–95	90	88–92	92	89–94
ARC	85	73–95	91	84–94	88	84–92	90	86–94
5F	91	80–98	93	90–95	92	89–94	94	91–96

Table 2 Dose volume histogram analysis for PTV (%)

Technique	V95		D95		D50		D5	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
4F	85	73–95	87	82–91	86	80–90	88	82–92
6F	85	74–90	87	82–91	86	82–91	88	82–93
ARC	85	73–95	86	80–91	86	80–92	88	82–92
5F	91	80–98	91	89–93	90	88–91	91.4	90–93

Table 3 Dose volume histogram analysis for organ at risk/normal tissues (%)

Organ	Parameter	4F technique		6F technique		ARC		5F technique	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
Rectum	V95	13	10–16	0	0	0.5	0–1	1	1–2
	V50	91	83–93	76	71–82	79	71–83	70	64–73
	DM	55	53–57	51	48–56	51	49–54	51	50–54
Urinary bladder	V95	16	10–21	4	2–10	14	10–16	41	2–9
	V50	38	35–40	25	20–30	43	35–50	24	20–29
	DM	56	51–61	51	49–51	51	50–54	51	50–54
Femora heads	V95	0	0	0	0	0	0	0	0
	V50	1	1–3	8	3–11	0	0	3	0–6
	DM	28	25–32	35	32–39	29	24–31	34	32–37

as compared to when planning was done by fixed field technique (0%, 0% and 29% versus 0%, 1% and 28%).

Akazawa *et al* [8] compared five different three-dimensional techniques for treatment of prostate without seminal vesicles using 4F and 6F conformal fields, open and blocked 120° bilateral arcs. They analyzed dose volume histograms and revealed that use of blocked arcs significantly improved the dose distribution compared to using standard arcs and 4F conformal techniques.

Cattaneo and colleagues [9] used various fixed fields techniques (three fields, four fields with wedges and without wedges, six fields with wedges and without wedges) for plan evaluation. They concluded that there is no fixed field technique absolutely better than other technique because if one technique gives the best sparing of rectum the other gives best sparing of urinary bladder, similarly the femoral head sparing was found to be the best in another technique. It was also found that rectum is the dose limiting organ at risk (OAR) for almost all techniques.

From the comparison of the four techniques, arcs spare the femoral heads the greatest due to decreased weighting through the lateral projection, along with the use of conformal blocking and 5F technique spare more than other techniques due anterior field and all arrange fields are oblique.

All the treatment plans were also evaluated using dose volume histograms (DVHs), which are plots of cumulative dose volume distribution and graphically summarize the simulated radiation distribution within a volume of interest of patient, which would result from a proposed radiation treatment plan. DVHs show promise as tools for comparing rival treatment plans for a specific patient by clearly presenting the uniformity of dose in the target volume and any hot spots in adjacent normal organs and tissues.

It was observed in the study that 5F technique gave a far better target coverage than any other techniques however statistically no significant difference was noted for GTV and CTV coverage by the two techniques but statistically significant difference was noted for PTV coverage.

During radiation treatment planning, doses to surrounding normal tissues/organs at risk i.e. V95, D50 and Dm should be kept to minimum. From this point of view the four techniques of 4F, 6F, full rotational and 5F were analyzed and compared statistically. There was a significant difference between skip-scan technique and any other techniques as far as dose delivery to OAR like rectum and urinary bladder was concerned. In the case of femoral heads, representing the normal tissues at a distance from

target volume, received less dose by full rotation (360°) 5F technique as compared to fixed field techniques. Particularly radiation exposure sparing by rotation/skip scan technique to the distant normal tissues was found to be statistically significant ($P < 0.05$).

Conclusion

Of the different arrangements conformal plans with 3 fields techniques, 5F technique is a better optimum plan as compared to the other standard chosen fields. For the iso-dose distribution for different fixed fields/rotational, 5F technique was meticulously compared on each CT slice of each patient. It was found that in all cases tumor volumes could be covered by 100% iso-dose lines when planned by any type of 3D planning technique. However, the sparing pattern obtained by 5F technique was far better than iso-dose pattern obtained by other techniques.

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Clinical study about endoscopic inguinal lymphadenectomy for patients with vulvar carcinoma

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Abstract ***Objective:*** The aim of this study was to explore the new method of inguinal lymphadenectomy in order to reduce side effects of conventional method for patients with vulvar carcinoma. ***Methods:*** Lipolysis and liposuction were performed to subcutaneous fat on inguinal region. We inserted endoscope and filled with CO_2 gases to this field and then resected inguinal lymph nodes with ultrasonic scalpel. The operative field was placed with the vacuum sealing drainage and pressured with soft saline bag after the operation. ***Results:*** Many lymphatic vessel, small blood vessels and hanging lymph nodes in the subcutaneous tissues of inguinal region were revealed after lipolysis and liposuction and lymph nodes can be easily removed. The follow-up so far showed that healing of the incision was good and there was no lymphedema of patient's lower limb and inguinal region. ***Conclusion:*** Endoscopic inguinal lymphadenectomy can resect the lymph nodes and keep most of the lymphatic vessels. So this technique has less influence on the lymph backflow of lower limb and inguinal region and can avoid the huge incision of conventional method. This method is worthy of further study.

Key words vulvar neoplasms; lymph node; laparoscope

The endoscopic surgery has been used in gynecology for many years, and has been applied to many diseases of gynecology. In some benign tumors and lesions of gynecology, laparoscopic surgery has the dominant status, such as ovarian cyst, ectopic pregnancy etc. The endoscopic surgery have many characteristics such as small incision, healing fast, beautiful appearance of incision because of better healing of skin and mucosa, small influence on visceral function.

The inguinal lymph nodes of patients with vulvar carcinoma are the common sites with metastatic lesion. The inguinal lymphadenectomy is necessary in radical vulvectomy because we can reach the purpose of radical excision and judge if there are metastases only by the removal of inguinal lymph node, and can provide evidence of subsequent radiation therapy. The conventional method of inguinal lymphadenectomy have many defects, such as long incision, healing difficulties of incision, apparent pit of the surgical site after healing and adverse influence on lymph circulation after the operation. Some patients after the operation may appear some serious problems, such as delay healing of the incision, lymphedema of lower limbs (commonly known as the elephantiasis), and so on. So we carried out inguinal lymphadenectomy by endoscope.

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Materials and methods

Patients

We selected two inpatients with vulvar carcinoma on Department of Gynecologic Oncology of Jiangsu Cancer Hospital as objects.

Case 1: 45 years old, was admitted to the hospital on 11 April 2011, and underwent the operation on April 19. The patient was done the biopsy of vulva mass on other hospital because she had a vulva mass for four months and ulcer of the mass for one month. Pathological examination after the biopsy demonstrated VINIII with infiltration of cancer cell. By physical examination, we found there were multiple palpable swollen lymph nodes with on the left groin, and diameter of the maximal node was 2 cm. There was a mass with burst surface and mobile fundus on the upper part of left labia minora, and its diameter was 4 cm. Preoperative CT examination results showed that diameter of the maximal left inguinal lymph node was 1.7 × 1.3 cm and the vulva was obviously incrassate, according with the signs of vulvar cancer (Fig. 1). Large

pelvic lymph node was not found by CT examination.

Case 2: 55 years old, was admitted to the hospital on 23 May 2011, and underwent the operation on May 28. The patient was underwent the resection of the tumor vulvae in the outer court due to a mass on the right vulva that had been found for 5 years and had a notable enlargement recently for one month. Postoperative pathological report after consultation by The Department of Pathology of Jiangsu Cancer Hospital showed that it accorded with the hidradhomacarcinoma of right vulva. By physical examination, we found there were no obvious palpable swollen lymph nodes with on both sides of groin and was a visible scar with subcutaneous scleroma about $2 \times 0.5 \times 0.5$ cm in size on the upper part of right labia minora. The fundus of the scleroma was mobile. Preoperative CT examination did not find obvious swollen lymph nodes on both sides of the groin and pelvic cacity.

Methods

We took the groin area as surgical site, with its upper bound to 3 cm above the inguinal ligament, lower edge to 10 cm under the inguinal ligament, outside bound to the inside of anterior superior iliac spine, inside bound to the outside of pubic tubercle. We punctured respectively to the subcutaneous fat tissue by the long needle at the upper lateral party and inferior party of the operative field after disinfecting and outlining by sterile towels and injected the de-fatting liquid consisted of sterilization distilled water 250 mL, 0.9% saline water 250 mL, 2% lidocaine 20 mL and 0.1% adrenaline 1 mL [1]. Liposuction was performed after 15 to 20 min after the lipolysis. After cleaning the subcutaneous fat of the operative field, we punctured to subcutaneous tissue and inserted the trocars respectively in the outside, upper lateral side and inferior lateral side of the operative field. CO_2 gases were filled to the operative field and its pressure was set to 8 mmHg. After observing the distribution of lymph node inside the cavity, we separated and cut off the araneose fat interval, part of the small vessel and lymphatics in the cavity by ultrasonic scalpel. Small lymph nodes can be directly resected and taken out through the 1 cm trocar. Lymph nodes larger than 1 cm after cutting off the connected small lymphatics and vessel can be taken out directly with allis clamp after pulling out 1cm trocar or be taken out after expanding incision nearly to the end of the operation (Fig. 2–5). After the resection of inguinal lymph nodes, a pediatric stomach tube which had been cut to be porous was placed in the operative field as a drainage tube through the puncture hole in the inferior party of the operative field. Each puncture hole was dealed with intradermal suture by absorbable sutures. After the operation, the drainage tube was connected to a continuous vacuum suction device. Postoperative operative field was oppressed with a saline soft bag.

We adopted the new staging criteria of vulvar cancer by FIGO in 2009 as the basis of postoperative operation-pathologic stage [2].

Results

Four inguinal lymph nodes were resected respectively on both sides of the groin area of Case 1. There was a swollen lymph node on the left side and intro-operative pathological report by frozen section pathologic examination after resection showed that it accorded with metastatic squamous cell carcinoma. Postoperative routine pathological examination for the left vulvar mass 3.5×3.1 cm in size demonstrated squamous cell carcinoma of G I–II level. Dermis and subcutaneous fat were involved and there were no residual cancer cell around the surgical margin and basal parts. Positive inguinal lymph nodes of left and right side were 1/4 and 0/4 respectively. The postoperative operation-pathologic stage was IIIa stage. There was not skin necrosis and change of skin color on the groin area after operation. Healing of incision was good and the groin area was slightly sunk. The volume of drainage liquid was 10–40 mL daily on the first to five day after operation and decreased since the six day. The drainage tube was pulled out on the thirteen day when the volume of drainage liquid was below 5 mL. The patient underwent a supplementary radiation and chemotherapy treatment after the operation. The follow-up so far showed that healing of the incision was good and there was no swelling of her lower limb.

Four inguinal lymph nodes were resected on the right side of the groin area of Case 2. Postoperative pathological examination of these four nodes revealed chronic inflammation change. Pathological examination of removing vulvar tissue showed foreign bodies (stitches), infiltration of inflammatory cells and a few amount of infiltrated cancer cells in the fibrous tissue. There were no residual cancer cell around the surgical margin and basal parts. The postoperative operation-pathologic stage was Ib stage. There was not skin necrosis and change of skin color on the groin area after operation. Healing of incision was good and the groin area was slightly sunk. The volume of drainage liquid was 50 mL daily on the first to two day after operation and decreased since the three day. The drainage tube was pulled out on the eight day when the volume of drainage liquid reduced to 5 mL. This patient did not need to receive a supplementary chemoradiotherapy.

Discussion

Conventional method of inguinal lymphadenectomy for female patients with vulvar cancer has a huge surgical wound. It has a longitudinal incision which across the

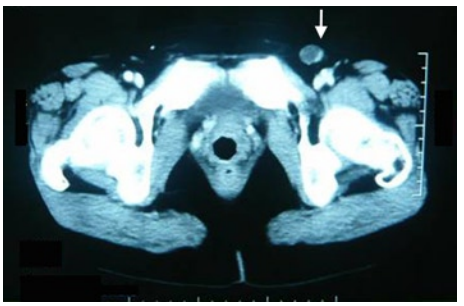

Fig. 1 CT picture of the case 1, the arrowed was the left swelling groin lymph node

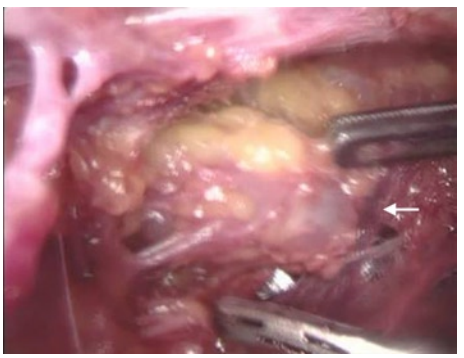

Fig. 2 The left swelling groin lymph node (positive node, appeared after lipolysis and liposuction and filled with CO_2 gases)

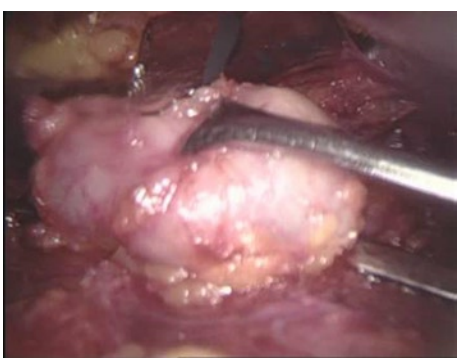

Fig. 3 The left swelling groin lymph node (positive node)

midpoint of groin ligament and its length may reach to 15 cm. This method should resect subcutaneous fat tissues containing lymph nodes in the areas around the groin ligament up and down to 15 cm which inside reaches to the pubis nodules and outside reaches to the anterior superior iliac spine.

Lipolysis and liposuction are fully performed to subcutaneous fat on inguinal region in the endoscopic inguinal lymphadenectomy and the fatty tissue of this region is removed. Only the fat intervals, lymphatics and "hanging" lymph nodes are kept (Fig. 2–5). Then the lymph nodes are easily resected one by one.

Small incision can be dealed with intradermal suture

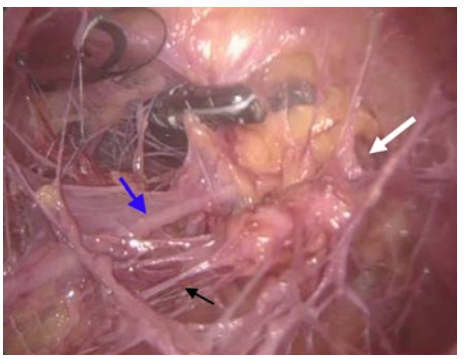

Fig. 4 Negative lymph node (white arrow), lymph vessel (black arrow) and small blood vessel (blue arrow)

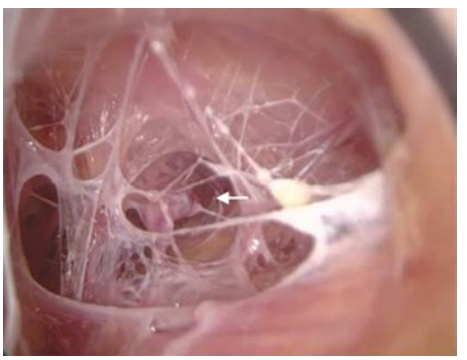

Fig. 5 Small groin lymph node

by absorbable sutures and form line kind scar after healing. After the operation, the drainage should be set up and the operative field should be oppressed with a saline soft bag in order to close the cavity of the wound. Drainage liquid may disappear generally within 7 to 10 days. The incision can be primary healing and there is small influence on the lymph backflow of lower limbs.

Lipolysis and liposuction fully to the operation region have been commonly used in the endoscopic axillary lymphadenectomy [1], but this technique is rarely used in the inguinal lymphadenectomy for female patients with vulvar cancer. Now reported laparoscopic inguinal lymphadenectomy was performed by directly inserting the laparoscope to the subcutaneous tissue of abdomen through small incision on umbilical plexus and reaching the groin area. Lymphatic and adipose tissue of the inguinal region were cleared after filling CO_2 gases to the operative field. The scope of surgical resection by this method was similar to the conventional method. The effectiveness of this method was good on the basis of report [3]. We applied the method of endoscopic axillary lymphadenectomy to the treatment of female patients with vulvar cancer and achieved good effect. First and key step of this method were lipolysis and liposuction fully to subcutaneous fat tissues of the operation region that can be beneficial to fill CO_2 gases to the operative field

and for the exposure of operative field. In the meantime, this step may avoid that the operation region become rugged after operation. Hypotonicity of de-fatting liquid can make fat cells swelling and broken. This is beneficial to the liposuction. Vasoconstriction property of adrenaline in de-fatting liquid can obviously reduce the amount of blood loss in the process of liposuction and operation and this will be helpful for the display of surgery field and the perform of the operation. A metal suction tube with lateral holes connected to a continuous vacuum suction device can be used in the process of liposuction. The pressure should be kept from 0.04 Mpa to 0.08 Mpa in order to control easily the suction tube and avoid damage to the skin and blood vessels in the process of liposuction.

Comparing with the conventional method of inguinal lymphadenectomy, endoscopic inguinal lymphadenectomy in this study do not need to resect too much subcutaneous fat tissues and lymphatics in the fat tissues which are responsible for the drainage of lymphatic fluid of lower limb. After lipolysis and liposuction, we only need to cut off the lymphatics connected to lymph nodes and resect exposed lymph nodes. The operational injury of this method is relatively small. Comparing with the conventional method, endoscopic inguinal lymphadenectomy does not increase the risk of tumor metastasis [4]. In the meantime, endoscopic inguinal lymphadenectomy has relatively less influence on the lymphatic backflow and function of the lower limb. So we think this method is worthy of further study.

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Assistant radiotherapeutic effect of Jiaqi Mixture on tumor-bearing mice

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Abstract ***Objective:*** The aim was to study the assistant radiotherapeutic effect of Jiaqi Mixture (JQM) on Ehrlich's ascites carcinoma (EAC) mice. ***Methods:*** The EAC-cancer model was made up with Kunming mice. The tumor-bearing mice were treated with whole body exposure (8 Gy) and intragastric administration of JQM, and the changes of tumor weight, the total number of white blood cells (WBC) and immune system were observed. ***Results:*** The average tumor weight, WBC, spleen coefficient, the stimulation index (SI) of Con A and LPS and the natural killing (NK) cell activity of mice decreased in some degree after radiotherapy, but the average tumor weight decreased more obviously in radiotherapy + medicine groups (compared with tumor control group, $P < 0.05$); and the other above indexes were much higher in radiotherapy + medicine groups than those in radiotherapy groups ($P < 0.05$–0.01). ***Conclusion:*** It was suggested that JQM can enhance the effect of radiation therapy and protect the normal immune system caused by radiation therapy.

Key words Jiaqi mixture; radiotherapy; blastisation of lymphocyte; NK cell activity; radioprotection

Jiaqi Mixture (JQM) is a compound preparation, consisting of the traditional Chinese drugs (Raidx Astragali, Radix Acanthopanacis Semticosi, etc) can support healthy energy, boost Qi and invigorate the spleen. The clinical application study of the JQM compound preparation has been carried out for many years in Tongji Hospital. It can be mainly used for enhancing patients' immunity and assisting to treat the tumor diseases. There are many reports that the drug and its prescription components can counteract tumor and enhance immunologic competence in recent years [1–3]. In this report, we have investigated that the synergy and protection effect of JQM on radiotherapy in tumo-bearing mice.

Materials and methods

Main reagents and instruments

JQM was provided by Department of Pharmacy, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (Batch No.: 20060908), 2.2 g crude drugs in 1 mL JQM. The medium RPMI1640, Concanavalin (ConA), Lipopolysaccharide (LPS) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from GIBCO (USA).

Complete culture solution: The medium RPMI1640, 10% Solcoseryl, 1% Glutaminate (200 mmol/L), Penicillin(100 U/mL), Streptomycin (100 μg/L), 2-mercaptoethanol (5×10^{-5} mol/L), 3121 model CO_2 incubator (Forma Company, China), 450 Eliasa (Awareness Company, USA).

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Animals

Kunmimg male mice, were supplied by Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology. One mouse with Ehrlich's ascites carcinoma (EAC) was obtained from Tumor Laboratory of Tongji Medical College, Huazhong University of Science and Technology. The mice can be used after 7 days when they were inoculated EAC in abdominal cavity.

The YAC-1 cell line was from Shanghai Institute of Cell Biology, Chinese Academy of Sciences.

Tumor modeling

Three mL ascites were drawn from the mouse with EAC aseptically and diluted with normal saline 30 mL (about 1×10^7 cells in 1 mL), every mouse was inoculated 0.2 mL in right axillary space subcutaneously [4].

Grouping and treating

The mice were divided into 4 groups, the tumor control group, radiotherapy group and radiotherapy + JQM group

Table 1 The effect on weight of tumor and spleen coefficient ($\bar{x} \pm s$, $n = 10$)

Group	Dosage	Weight of tumor (g)	Spleen coefficient
Control		1.03 ± 0.18	0.20 ± 0.021
Radiotherapy	8 Gy	0.86 ± 0.13*	0.13 ± 0.038**
Radiotherapy + JQM	8 Gy + 13.75 g/Kg	0.82 ± 0.27*	0.17 ± 0.026*△
Radiotherapy + JQM	8 Gy + 27.5 g/Kg	0.82 ± 0.23*	0.18 ± 0.031*△

Compared with the tumor control group, * $P < 0.05$; Compared with the radiotherapy group, △ $P < 0.05$

Table 2 The effect on blood cell count ($\bar{\chi} \pm s$, $n = 10$)

Group	Dosage	WBC (10^9/L)	RBC (10^{12}/L)	BPC (10^{14}/L)
Control		6.84 ± 1.22	8.64 ± 1.86	5.40 ± 0.39
Radiotherapy	8 Gy	2.62 ± 0.41**	6.58 ± 1.17	5.09 ± 0.53
Radiotherapy + JQM	8 Gy + 13.75 g/Kg	3.37 ± 0.33**△	6.25 ± 1.54	5.24 ± 0.42
Radiotherapy + JQM	8 Gy + 27.5 g/Kg	3.71 ± 0.45**△	6.72 ± 0.88	5.06 ± 0.76△

Compared with the tumor control group, * $P < 0.05$; Compared with the radiotherapy group, △ $P < 0.05$

Table 3 The effect of JQM on radiotherapy in tumor-bearing mice ($\bar{x} \pm s$, $n = 10$)

Group	Dosage	ConA (SI)	LPS (SI)	NK cells activity (%)
Tumor control		2.32 ± 0.16	2.02 ± 0.03	49.3 ± 7.9
Radiotherapy	8 Gy	1.46 ± 0.14**	1.27 ± 0.16**	24.5 ± 7.5**
Radiotherapy + JQM	8 Gy + 13.75 g/Kg	2.09 ± 0.10*△△	1.91 ± 0.14△△	32.2 ± 6.1*△
Radiotherapy + JQM	8 Gy + 27.5 g/Kg	2.20 ± 0.23△△	1.89 ± 0.13△△	39.2 ± 9.4*△

Compared with the tumor control group, * $P < 0.05$, ** $P < 0.01$; Compared with the radiotherapy group, △ $P < 0.05$, △△ $P < 0.01$

(2 different doses). Every group was received JQM, and inoculated tumor cells on the 3rd day. All the mice were irradiated by Co-60 once on the 6th day and 10th day except the tumor control group, the irradiation dose was 4 Gy; and all the mice were killed on the 16th day. Striped and weighed tumor and spleen, determined blood, spleen lymphocyte transformation degree and the activity of NK cells [5–7].

Splenic lymphocyte proliferation assay

Put the spleen into dish filled with Hanks solution and triturate it into single cell suspension under the condition of asepsis. Filtered from 200 screen mesh and washed twice with Hanks solution, then centrifuged 10 min (1000 r/min). The cells were cultivated in complete culture solution and the density is 3×10^6 cells/mL. The cells were divided into 3 wells and plated into 24-well plates, one for control, the others were with LPS or ConA, cultivated at 37 ℃, in an atmosphere of 5% CO_2 for 72 h. At the time of the last 4 h, remove the supernatant solution 0.7 mL and add RPMI164O without solcoserly and MTT 50 μL. By the end, add acidity isopropanol 1 mL. OD were determined by Eliasa at 570 nm.

Determination of NK cell activity

Spleen cell suspension was made at a density of 1×10^7 cells/mL as effector cell and YAC-1 cell as target cell at a density of 1×10^5 cells/mL. They were planted into 24-well plates, each setting up 3 wells with 2 other control groups: (1) 0.1 mL target cells + 0.1 mL 1640; (2) 0.1 mL target cells + 0.1 NP at 37 ℃, in an atmosphere of 5% CO_2 for 2 h. Remove 0.1 mL supernatant of each well to another plate at 37 ℃. After 10 min, add fresh LDH solution 0.1 mL for 15 min, ending it with citric acid.

Statistical analysis

Data were presented as mean ± SD. *t*-test was used to identify significant differences between groups and statistical significance was set at $P < 0.05$.

Results

General animation

The weight of the mice decreased when they were inoculated tumor cells. In the late period of the experiment, the mice in the tumor control group and radiotherapy group were anorexia and their color pattern were murky gray, but the mice in the radiotherapy + JQM group were active and their color pattern were gloss.

Weight of tumor and spleen coefficient

In the radiotherapy group, the weight of tumor decrease slightly; but in the radiotherapy+ JQM group, the weight of tumor decreased obviously (compared with the tumor control group, $P < 0.05$). Spleen coefficient declined obviously after radiation treatment (compared

with tumor control group, $P < 0.05$), but the spleen coefficient in radiotherapy + JQM group were higher than that in radiotherapy group, $P < 0.05$. This results showed that JQM possessed the protective effects on injured spleen by radiation.

Blood cell count

The changes in blood cells between tumor control group and radiotherapy group shew that the white blood cell count were sharply declined after radiotherapy, $P < 0.01$. The white blood cell count of radiotherapy + JQM group showed a certain degree recovery, compared with the radiotherapy group, $P < 0.05$.

Degree of the blastisation of lymphocyte and NK cells activity

In the radiotherapy group, the SI of ConA and LPS decreased obviously, compared with the tumor control group, $P < 0.01$. The SI in the radiotherapy+ JQM group is higher than that in radiotherapy group, $P < 0.01$. The NK cells activity of mice reduced obviously after radiotherapy (compared with the tumor control group, $P < 0.01$–0.05), but the NK cells activity in the radiotherapy + JQM groups stronger than that in radiotherapy group ($P < 0.05$).

Discussion

Traditional Chinese medicine (TCM) believes that tumors occur due to the deficiency of righteousness, evil poison staying and gathering in body. It is a commonly method used in clinic to inhibit tumor growth or metastasis by aiding righteousness and firming foundation [8]. That is to say, by replenishing" spleen" and "qi" the immune function and marrow hematopoietic function could be improved and the metabolism could be promoted. Surgery, radiotherapy, and chemotherapy were the main therapeutic methods for malignant tumor, and About 50%–70% of patients with malignant tumor received radiotherapy [9]. Radiation therapy usually leads to decreased immune function, bone marrow suppression and other side effects. The obvious symptom of side effects such as fatigue, poor appetite, leukopenia, etc. could leads to interrupt treatment or extend treatment time. Therefore, some TCM that possessed replenishing "spleen" and "qi" efficacy can reduce the toxic side effects during radiation therapy, improve patient tolerance, shorten radiotherapy time and have a good auxiliary therapy effect.

TCM astragalus can replenish "qi" and be used for fatigue and fastidium. As a kind of active ingredient in Astragalus, astragalus polysaccharides (APS) has a wider anti-cancer effect, either can kill the tumor cells, but also can enhance immune function, reduce the adverse reaction caused by radiotherapy and chemotherapy [10]. Astragalus as a main drug has been extensively involved anti-cancer TCM prescription. TCM acanthopanax can replenish "qi" and invigorate "spleen", be used for poor appetite, loose stools, fatigue etc. Recently, acanthopanax as a subsidiary of anticarcinogenic drugs has been widely used in clinic, especially in radiotherapy and chemotherapy for tumor [11]. JQM as a hospital preparation was made from two TCM Astragalus and acanthopanax, and has been use for adjuvant therapy of tumor in radiotherapy and chemotherapy for a long time, meanwhile, it can also be used for deficiency-weakness of spleen-qi. This experiment shows that JQM can enhance the antitumous effect of radiotherapy, and can also protect the immune system from radiotherapy. After radiotherapy, the mice reflected the symptom of lassitude, anorexia and hypofunction of immune system; all of these are the appearance of deficiency of spleen-qi in traditional Chinese medicine. The results of this experiment hint that some Chinese drugs with the effect of nourishing "qi" and invigorating spleen have the function of protecting the immune system from radiotherapy.

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Clinical significance of MDM2 as a tumor biomarker*

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Abstract MDM2 (the product of murine double minute 2 gene) is one of the most important negative regulators of p53, which can binds to p53 and initiates ubiquitin-mediated degradation. Besides, MDM2 also controls the activity of several cell-cycle regulators, e.g. pRB, p21, E2F1, independently of p53. The important role of MDM2 in cell-cycle regulation indicates it to be a point of interest in cancer research. In recent years, studies revealed that MDM2 participated in the genesis and development of human tumors. The expression levels and activity of MDM2 was associated with the invasion, metastasis, prognosis and more practical, chemosensitivity. MDM2 is becoming a novel biomarker in cancer prognosis and chemosensitivity prediction.

Key words oncogene ; MDM2; p53; predictive biomarker

Murine double minute 2, MDM2, function as an oncogene, with its transcription product protein MDM2, is an important negative regulator of p53, the transcription product of oncogene TP53. MDM2 participates and has an important role in regulating of cell proliferation and apoptosis through both the p53-dependent and p53-independent pathway. And research results showed that protein MDM2 was also very important to carcinogenesis and cancer progression. In recent years, it has been confirmed that mutations, amplification and overexpression of MDM2 in various human malignancies. And abnormal gene expression or dysfunction of MDM2 can change the biological behavior, tumor susceptibility and prognosis of tumor differently, and can also affect tumor response to treatment in variant degrees. In this review, by summarizing relevant literatures on MDM2 and MDM2 published in recent years, we give a brief introduction of the biological function of MDM2, and then focus on the potential clinical significance of MDM2 as molecular markers for cancer susceptibility, tumor prognosis and response to treatment.

Biological function of MDM2

It has been confirmed that protein p53, is one of the most important elements in oncogenesis. It can inhibit cell proliferation and promote cell apoptosis by regulating cell cycle arrest, cell apoptosis and repair of DNA damage. Functional status of TP53 has influence on cancer prognosis and sensitivity to chemotherapeutic drugs.

In kinds of human tumors, there are functional inactivation of p53, caused by gene mutation, missing of positive regulatory factors, methylation in promoter region of TP53, or overexpression of MDM2 and MDM2 family members. MDM2, as an important negative regulator of TP53, is an important component of the p53-dependent signal transduction pathway, and plays an important role in tumorigenesis and development. Under cell stress conditions, such as hypoxia, DNA damage, oncogene activation, TP53 is activated. Its downstream gene, MDM2, is activated at the same time. TP53 activation starts different signal transduction pathways in cell cycle, and rises intracellular expression of MDM2, while the later leads to the downregulation of TP53. A negative feedback loop between TP53 and MDM2 can regulate the intracellular expression level of both proteins precisely.

Now, we know that MDM2 can regulate the function of p53 in different ways [1]. It can inhibit downstream gene transcription of TP53 by combining directly to p53, block the interaction of p53 and its transcription factors. It can also mediate the transport of the MDM2-combined p53 from nucleus to cytoplasm, then MDM2 function as an E3 ubiquitin enzymes, causing ubiquitination and degradation of p53. Except for p53, MDM2 also has complicated interaction with a variety of important cell cycle regulatory factors [2], such as pRB, p21, E2F1. MDM2 regulates

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function and promotes degradation of phosphorylated retinoblastoma protein (pRB) and p21 and interacts with E2F1, which leads cell cycle into S phase. Thus, MDM2 can promote cell proliferation in more than one way both in normal and tumor cells.

MDM2 SNP309 and cancer susceptibility

Bond *et al* [3] found a single nucleotide polymorphisms (SNP), MDM2 SNP309 (rs2279744 T/G) in P2 promoter area of MDM2 in 2004. When the SNP loci were G genotype, the affinity of MDM2 P2 promoter to transcription factor SP1 were enhanced, promoting transcription of MDM2, increase MDM2 expression level in tumor cells. Then the later weaken the tumor inhibition function of the p53-dependent pathway, which further promote the genesis and earlier occurrence of a variety of sporadic tumors.

Hu *et al* [4] did a meta-analysis on genotype distribution of MDM2 SNP309, by analyzing overall 14,770 cases of cancer patients and 14,524 cases of normal controls. The result show that GG genotype can significantly increase the risk of cancer occurrence (OR 1.17), which prompted that it may assist to determine cancer susceptibility by genotyping MDM2 SNP309. When stratified by tumors location, the results showed that MDM2 SNP309 was related to increased cancer susceptibility of lung cancer [4, 5], gastric cancer [6], esophageal cancer [7], nasopharyngeal carcinoma [8], liver cancer [9], bladder cancer [10], but not to breast cancer or colorectal cancer [4, 11]. And it also shows that there are some ethnic differences about the relationship of MDM2 SNP309 and cancer susceptibility. The study carried out by Gui [5] *et al* that included 6063 cases of lung cancer, demonstrated that GG genotype of MDM2 SNP309 increased the risk of lung cancer about 17% in Asian populations, but does not increase the risk of lung cancer in European and African areas. At about the same time, another research reported that GG genotype in MDM2 SNP309 is a risk factor of breast cancer in Chinese female, but not in non-Chinese women [12].

Smoking has also certain impact on relationship of MDM2 overexpression and cancer susceptibility. In view of the fact that smoking has influence on incidence, histological type and treatment sensitivity of lung cancer, research groups were further classified as heavy or light smokers, never-smokers or quitters, the research results revealed that the relevance of MDM2 SNP309 and the risk of lung cancer was changed in different groups. MDM2 SNP309 GG genotype increases the risk of non-small cell lung cancer (NSCLC) in light smokers and never-smokers，but not in heavy smokers [13]. By the way, smoking is also a factor in many of esophageal cancer. Hong *et al* [7] genotyped MDM2 SNP309 of 758 cases of esophageal squamous cell carcinoma and 1420 cases of normal control, showed that the risk of esophageal squamous cell carcinoma in patients with GG genotype was 1.49 times higher than patients with TT genotype, and even higher (up to 5.29 times) for GG genotype subgroup patients who smoke.

MDM2 SNP309 and prognosis of tumor

Overexpression of MDM2 was detected in a variety of human tumors, and it was shown to be connected with tumor invasiveness and metastasis. Those research results suggested that MDM2 may be associated with tumor prognosis. Although, till now, researchers have no agreement on relationship between MDM2 over-expression and tumor prognosis, generally speaking, malignance with overexpressed MDM2, are more aggressive, more likely to metastasize and to have poor prognosis. Here, we further discussed the prognostic role of MDM2 in different kinds of primary tumors.

Lung cancer

Overexpression of MDM2 was shown to closely related with clinicopathological features of lung cancer, such as differentiation, tumor stage and lymph node metastasis. Expression of MDM2 can be used as an important reference factors to estimate the degree of malignancy and metastatic potential of NSCLC. MDM2 expression is significantly higher than normal lung tissue [14]. Higher level of MDM2 was also detected in advanced lung cancer comparing to early stage lung cancer, patients with lymph node metastasis vs those without lymph node metastasis and poorly differentiated cancer vs. well-differentiated cancer. Heist *et al* [15] reported that early stage lung cancer, especially squamous cell carcinoma, with overexpressed MDM2, had poor prognosis. Han [16] and his colleagues focused on advanced lung cancer, and they identified that MDM2 SNP309 TT genotype carriers have significantly longer overall survival time (OS) than patients with TG or GG genotype (16.5 vs. 13.6 months). Thus, they took MDM2 SNP309 TG/GG genotype as a predicted factor for short OS. Other researchers, like Chien [17], focus on stage I lung cancer. By studying 306 cases of stage I lung cancer, they reported that patients with both mutation of TP53 and overexpression of MDM2, have shorter survival time, while, patients with wild type P53, who also have MDM2 overexpression, had longer survivals than the others. Based on above studies, we presume that combination of mutation detection of TP53 and expression level examination of MDM2 can predict the prognosis of lung cancer more reliably.

Breast cancer

For breast cancer, MDM2 overexpression, is also re-

lated to poor prognosis. In about 38% of breast invasive ductal cancers, MDM2 was over-expressed, the rate was even higher in patients whose tumor are larger than > 2 cm in diameter ($P = 0.013$) [18]. Patients with GG genotype is younger than TT and TG genotype, with an average age of 47 years old ($P = 0.0067$, vs. TT/TG genotype 53.6/52 years old separately), and the GG genotype were more likely to have larger tumor size and higher rate of lymph node metastasis [19].

Turbin *et al* [20] detected the expression level of MDM2 on two batches of breast cancer patients (more than 2,000 patients). In the first cohort of 362 cases of breast cancer, there are about 14% (49/362) overexpression of MDM2 were detected, and the 10-year disease-free survival rate of the 49 patients was notably lower than the patients with low level of MDM2 (61% vs. 73%). In the second batch of 1747 cases of patients, 230 cases was identified with overexpression of MDM2, and the 10-year disease-free survival rate of the 230 patients was also significantly lower than the rest (58% vs. 73%, $P < 0.0001$). Further multivariate analysis were carried out, parameters including MDM2 expression, tumor differentiation, lymph node metastasis, ER expression and tumor size were analyzed. MDM2 expression was considered as an independent prognostic factor for breast cancer patients enrolled in the study. So the researchers proposed that MDM2 overexpression can be used as an independent predictors for poor prognosis of breast cancer.

Malignant tumor of digestive tract

Studies have shown that MDM2 overexpression affects the prognosis of gastric cancer, colorectal cancer, esophageal cancer and liver cancer.

Gastric cancer

The rate of MDM2 overexpression was significantly higher in gastric carcinoma than pre-cancerous lesions. There was only about 20% of gastric cancer did have functional changes in MDM2 and/or p53. Gastric cancers with higher expression level of MDM2, are more likely to have lower histological grade, stronger ability of invasiveness with lymph node metastasis, suggesting that MDM2 can be assisted in determine the degree of malignancy of gastric cancer, the potential of lymph node metastasis and prognosis of the patients. The study carried out by Ohmiya [6], proved that patients with MDM2 SNP309 GG genotype have short OS in comparison with TT / TG genotype carriers, and they took MDM2 SNP309 as an independent predictor for poor prognosis of advanced gastric cancer.

Colorectal cancer

Wang [21] detected the expression level of MDM2 on 72 cases of colorectal cancer using immunohistochemistry(I HC), the result showed that MDM2 overexpression is related to venous invasion, lymph node metastasis and liver metastasis. The patients who already have tumor venous invasion, lymph node metastasis or liver metastasis are more likely to have overexpression of MDM2. Yu [22] detected expression levels of MDM2 on 93 cases of colorectal cancer by IHC, shows that patients with MDM2 overexpression have short survival.

Esophageal cancer

The rate of MDM2 overexpression in esophageal squamous cell carcinoma is more than 50%, significantly higher than normal esophageal mucosa and esophageal mucosa with atypical hyperplasia, and the overexpression of MDM2 is more common in patients with poorly differentiated tumor and the ones already have lymph node metastasis [7]. Other studies showed that esophageal squamous cell carcinoma with MDM2 overexpression had poor prognosis [23]. Those research results suggested that MDM2 overexpression may have a role in the initialization of esophageal squamous cell carcinoma, and improve its invasion and metastasis.

Liver cancer

The expression level of MDM2 and MDM2 mRNA are strikingly different in hepatocellular carcinoma (HCC), adjacent non-cancerous liver and normal liver tissue. In liver cancer specimens, MDM2 expressed significantly higher than the other two kinds of tissue. And the expression of MDM2 mRNA can be detected in liver cancer, but not in adjacent non-cancerous liver and normal liver tissue [24]. This research result is in agreement with the results of other domestic researches. Thus, we presume that the overexpression of MDM2 is closely related to the development of hepatocellular carcinoma. Beyond that, studies also showed that HCC with has poor prognosis [25].

To sum up, based on all the researches mentioned above, we can conclude that the expression level of MDM2 and MDM2 SNP309 were correlated with more than one kind of human malignance.

Present research status of MDM2 as a indicative mark for personlized tumor therapy

Personalized tumor therapy guided by biomarkers is the main development direction of the future tumor therapy. The biological function of MDM2 in cell cycle regulation and tumorigenesis, suggesting that MDM2 may have the potential to serve as a biomarker for individualized choose of cell cycle specific agents.

In vitro studies, overexpression of MDM2 affect the susceptibility of tumor cells to commonly-used anticancer drugs, such as alkylating agents and inhibitors of topoisomerase I and II (Topo I and Topo II). Liu's research [26] shows that the copy number of MDM2 is an independent predictor for increased sensitivity of the NCI-60 cells to alkylating agents and topoisomerase in-

hibitors. MDM2 SNP309 GG genotype and wild type P53 indicated increased sensitivity of tumor cells to alkylating agents and TOP I inhibitors. Nayak *et al* [27] reported that MDM2 SNP309 GG genotype were related to drug resistance of certain Topo II inhibitors, such as etoposide, mitoxantrone and Amsacrine. Kondo [28] reported that glioblastoma with overexpressed MDM2 were less sensitivity to etoposide and doxorubicine. Further mechanism research demonstrated that both gene amplification of MDM2 and MDM2 SNP309 GG genotype lead to intracellular high expression level of MDM2, then the highly expressed MDM2 lead to downregulation of intracellular Topo II, which further lead to decreased drug sensitivity to Topo II inhibitors [3, 27]. Some scholars found that the activity of Topo II can be stabilized by using certain RNAi method. Then, drug-resistance of some Topo II-related drugs could be reduced. In the year of 2000, Zhou [29] reported that acute lymphoblastic leukemia children with overexpressed MDM2 were more likely to have resistance to doxorubicine.

Currently, there are also some reports on relations of MDM2 and tumor radiosensitivity. Tu [30] reported that genotype of MDM2 SNP309 have certain impact on prognosis of oral squamous cell carcinoma and its sensitivity to postoperative radiotherapy. MDM2 SNP309 GG genotype carriers have short disease-free survival (DFS) and OS, and the same result was obtained when patients with advanced oral squamous cell carcinoma were further analyzed. This study also showed that carriers of SNP309 GG genotype were less sensitivity to radiotherapy. Shinohara *et al* [31] studied 96 cases of bladder cancer who received platinum-based chemoradiotherapy (CRT), showed that the survival rate of patients with GG or GT genotype is higher than the TT genotype group (P = 0.009), and MDM2 SNP309 GG or GT genotype is an independent predictor of better prognosis for the research group (P = 0.031). However, the underlying relationship of MDM2 and effects of radiotherapy needs further study.

In addition, there are experiments confirmed that small molecular antagonists of MDM2, for example Nutlin, can increase the sensitivity of lung cancer cells to radiotherapy [32], and one may get collaborative antitumor function by combined application of Nutlin and conventional anticancer drugs, like topotecan, doxorubicine, etc [33].

Prospect

With the deep understanding of the function and mechanism of MDM2, we gradually realize its importance to human malignancy. And with more and more research is being carried out on MDM2 and tumor genesis, prognosis and treatment, MDM2 is being accetpted as a new tumor marker. And just for this reason, researchers are trying to develop new anticancer drugs which target on MDM2-p53 interaction. For further research, prospective clinical studies will be planned to test and verify whether MDM2 and MDM2 SNP309 could be informational signs for clinical choice of certain chemotherapy reagents.

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MR imaging features of secondary breast lymphoma in a male patient: a case report and literature review

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Abstract We reported the rare case of an elderly man with secondary breast lymphoma (SBL) associated with magnetic resonance imaging findings. MR images demonstrated multiple well-defined masses in the left breast, with heterogeneous enhancement on dynamic contrast-enhanced sequences. The time signal-intensity curve rapidly increased during the initial rise phase and washed out during the delayed phase. The apparent diffusion coefficient (ADC) value was 0.649×10^{-3} mm^2/s. Maximum intensity projection (MIP) showed that the masses were distributed in the upper outer quadrant, in the axillary region and in the lower outer region of the left chest wall. The pathology confirmed the diagnosis of non-Hodgkin's lymphoma. The combination of morphological and kinetic features, as well as a significantly lower ADC value, are helpful in the diagnosis of breast lymphoma and its differentiation from breast cancer.

Key words secondary breast lymphoma (SBL); magnetic resonance imaging (MRI); diffusion-weighted imaging (DWI); pathology

Secondary breast lymphoma (SBL) is a rare condition that often occurs in conjunction with extranodal disease, with the breast involved secondarily as part of a disseminated process. Lymphomatous breast involvement is encountered even more rarely in the male [1]. A few case reports have described the magnetic resonance (MR) appearance of breast lymphoma [2–4], but none to our knowledge have described diffusion-weighted imaging (DWI) of breast lymphoma in detail. Herein, we reported the extremely rare case of a 54-year-old man with SBL and the associated MR findings.

Case report

A 54-year-old man presented with subcutaneous masses on his back in October 2010 and subsequent lumps in his breast and axillary region in May 2011. He was admitted to our hospital (The First Affiliated Hospital of Chongqing Medical University, Chongqing, China) for management. Sonography revealed multiple hypoechoic and isoechoic solid masses in the back and breast. Subsequent mammography showed multiple irregular masses and enlarged lymph nodes in the left breast, which were potentially malignant and therefore categorized as breast imaging and reporting data system category 4 (BI-RADS 4) (Fig. 1).

The patient submitted to breast and back MR imaging. Breast images were obtained on the 3.0T MR system (GE Healthcare Medical Systems, Milwaukee, WI, USA) using a dedicated, bilateral, four-channel breast coil. The patient was examined in the prone position.

MR imaging demonstrated a well-defined irregular mass (3.5 cm in size) in the upper outer quadrant of the left breast, which showed heterogeneous hyper-intensity on T2-weighted images (T2WI) and diffusion-weighted images (DWI) (Fig. 2a and 2b). The apparent diffusion coefficient (ADC) value measured at the region of interest in the tumor was 0.649×10^{-3} mm^2/s when a sensitizing diffusion gradient factor with b values of 0 and 800 s/mm^2 was applied. The mass displayed iso-intensity on precontrast T1-weighted images (T1WI) with heterogeneous enhancement on dynamic contrast-enhanced sequences (Fig. 2c and 2d). The time signal-intensity curve exhibited a rapid increase during the initial rise phase and

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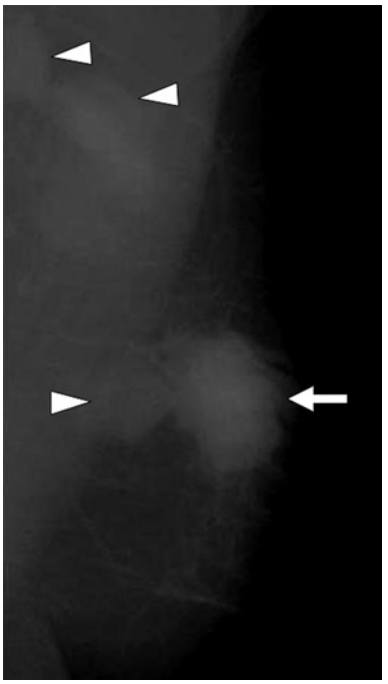

Fig. 1 A mediolateral oblique mammogram of the left breast showed a 3 cm × 2 cm irregular, indistinct mass (arrow) and a well-defined oval mass (arrowhead) in the upper outer quadrant of the left breast, which was associated with surrounding hypervascularization and axillary lymphadenopathy (arrowhead)

wash-out during the delayed phase. Reconstruction of the coronal-view maximum intensity projection (MIP) with subtracted images revealed multiple markedly enhanced masses distributed in the upper outer quadrant and the axillary region of the left breast as well as in the lower outer area of the left chest wall (Fig. 2e).

In addition, T2WI with fat suppression demonstrated the presence of multiple irregular high signal masses distributed diffusely in bilateral subcutaneous back tissue with heterogeneous enhancement on T1WI.

Conventional chest film and abdominal ultrasound examinations did not yield abnormal findings. Moreover, no evidence of disease was found in the bone marrow.

Fine-needle aspiration biopsy demonstrated the accumulation of small cells exhibiting atypical hyperplasia. Subsequently, the patient underwent modified radical mastectomy of his left breast. The pathological analysis confirmed the diagnosis of non-Hodgkin's lymphoma (NHL) classified as diffuse large B-cell lymphoma ($CD20^+$, Ki-67 70%$^+$, $CD10^-$, $CD3^-$, $Bcl\text{-}6^-$, $Bcl\text{-}2^-$, CK^-, ER^-, PR^-, $CerBb\text{-}2^-$) (Fig. 3).

Chemotherapy with CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone) was administered postoperatively. At the follow-up examination, the masses in the patient's back had disappeared almost entirely, and no occurrence or metastasis was found 5 months after the surgery.

Discussion

Breast lymphomas are rare conditions, which are nearly always NHL, accounting for approximately 2.2% of extranodal NHL and 0.15% of all breast malignancies [5, 6]. Most cases have been reported in women, with rare cases involving the male breast. The onset of breast NHL is typically experienced by patients sometime between the fourth and sixth decades of life [6].

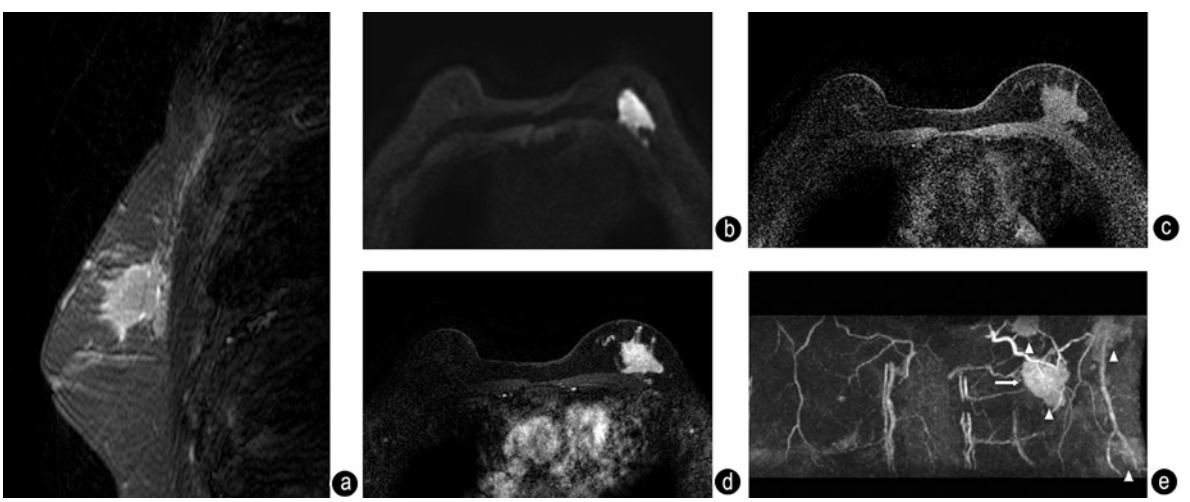

Fig. 2 Sagittal T2WI with fat suppression (a) and axial DWI (b) demonstrated an irregular hyper-intense mass and edema of the retromammary space. Axial precontrast T1WI with fat suppression (c) and contrast-enhanced T1WI (d) revealed heterogeneous marked enhancement of the mass. MIP in the coronal view (e) showed that the largest mass (arrow) was surrounded by multiple masses (arrowhead) in the upper outer quadrant, in the axillary region and in the lower outer region of the chest wall

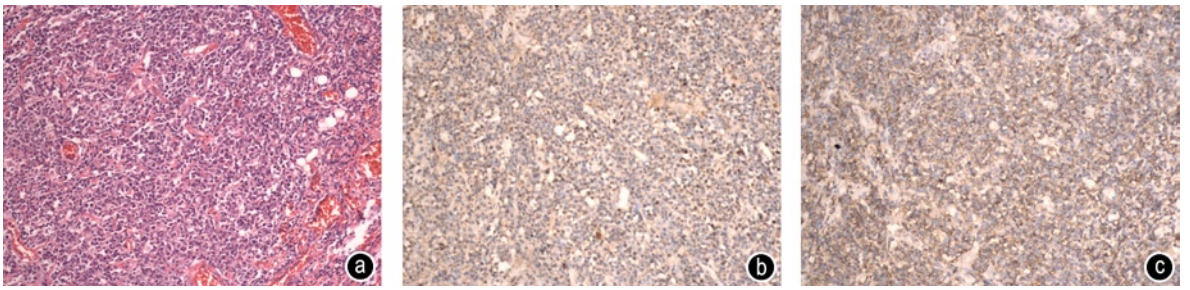

Fig. 3 (a) Microscopic findings showed numerous dense atypical lymphocytes containing medium-sized to large round nucleoli (hematoxylin and eosin, × 100). Immunohistochemical stainings for CD20 (b) and Ki-67 (c) were positive (magnification, × 100)

Primary breast lymphoma (PBL), as defined using the criteria by Wiseman and Liao [5], is defined as newly diagnosed NHL involving the breast with or without concomitant ipsilateral axillary lymph nodes. However, SBL is defined as lymphoma involving the breast with a systemic extramammary origin. According to these standards, the tumor in our patient was considered an SBL, which manifested as breast involvement of the lymphoma on his back.

The MR imaging characteristics of breast lymphoma have been reported in only a few cases [2–4, 7]. MR imaging demonstrated the presence of single or multiple well-defined, hypo-intense masses on T1WI and heterogeneous hyper-intense masses on T2WI. T1WI revealed a heterogeneously enhanced mass with a rapid initial increase in wash-in and wash-out or plateau kinetics on dynamic contrast-enhanced scans. The morphological and kinetic features of the mass may be helpful in the diagnosis of malignancy and the identification of benign solid masses such as fibroadenomas. In addition, the MIP technique is important for visualizing the full three-dimensional extent of breast involvement, which cannot be identified on conventional mammography and sonography. In the present case, the morphological features and the extent of mass extension to the breast and axillary regions, as well as the chest wall, which were fully demonstrated on MIP, can help in the differentiation of this tumor from breast cancer.

There are also a few articles describing the functional MR findings of primary NHL of the breast. Demirkan *et al* [3] have firstly reported the results of PBL on proton magnetic resonance spectroscopy, which demonstrated high levels of choline-containing compounds in the lesion. However, to our knowledge, this was the first case report of SBL in which DWI was performed and the ADC value was recorded.

Park *et al* [8] have reported that the mean ADC values (0.89×10^{-3} mm^2/s) of breast invasive ductal carcinoma are significantly lower than those of benign lesions and normal fibroglandular tissue when b values of 0 and 1000 s/mm^2 are applied.

Guo *et al* [9] have concluded that the low ADC value observed in breast cancer was mainly due to the effect of a high cell density; the mean ADC value was inversely correlated to tumor cellularity. Cellularity is known as an important index of tumor grade, and Ki-67 is a nuclear antigen that appears during the proliferative phase of the cell cycle. However, Yoshikawa *et al* [10] suggested that the mean ADC value was correlated with histological type rather than tumor cellularity.

In our case, the ADC value (0.649×10^{-3} mm^2/s) associated with lymphoma was significantly lower than that of breast cancer, which may be due to the higher degree of cellularity and cell hyperproliferation. Although ADC values in between those of lymphoma and breast cancer have not been statistically analyzed, the significantly lower ADC value of lymphoma is helpful in its differentiation from breast cancer. Certainly, differences between breast lymphoma and cancer in terms of ADC values should be investigated in more cases.

If the preoperative diagnosis of breast lymphoma had been suggested by either mammography or MRI and confirmed by core-tissue biopsy in this patient, the mastectomy would have been unnecessary and could have been avoided. Moreover, optimal treatment options such as chemotherapy alone or in combination with radiation could have been carried out in time.

In conclusion, rapid initial increases and washout kinetics may be detected on the dynamic contrast-enhanced MRI not only of carcinomas but also of non-Hodgkin lymphomas of the breast. The MIP technique is important for detecting multifocal masses and the extent of breast involvement, which cannot be determined by conventional mammography or sonography. Morphological and kinetic features as well as significantly lower ADC values are helpful in the diagnosis of breast lymphoma and its differentiation from breast cancer.

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An Iranian male with syringoid eccrine carcinoma misdiagnosed as basal cell carcinoma: a case report

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Abstract Syringoid carcinoma (syringoid eccrine carcinoma, or eccrine epithelioma) is a rare cutaneous tumor with some controversy regarding its correct definition. This tumor shows a slow growth and has often been for many years, some decades before diagnosis. It may also be difficult to differentiate from its benign counterpart (syringoma) or other adnexal carcinoma and cutaneous metastasis. There have been limited case reports of syringoid carcinoma in foreign literatures but none from Iran. Here we report a case of syringoid carcinoma in a 52 year-old Iranian man. Syringoid eccrine carcinoma is a very rare and uncommon diagnosed tumor thought to be derived from eccrine sweat apparatus. It locally invasive, destructive and often shows recurrence. It may also be difficult to differentiate from metastatic adenocarcinoma.

Key words adnexal carcinoma; syringoid; skin cancer

Melanoma skin cancers account for 4%–7% of all skin cancers, then non melanoma tumors for 93%–96%. Both basal cell carcinoma and squamous cell carcinoma represent > 80% of non melanoma skin cancers whereas benign and malignant adnexal tumors represent only 1%–2%, including mesenchymal, fatty and vascular tumors. Adnexal carcinoma of the skin drive from structures that have a common origin such as the apocrine and eccrine sweet gland, sebaceous glands and hair follicles. Malignant adnexal tumors are frequently located in the head and neck region but may appear on the fingers, toes, the trunk as well as the extremities [1–4]. Syringoid eccrine carcinoma is a very rare and uncommon diagnosed tumor thought to be derived from eccrine sweat apparatus. It locally invasive, destructive and often shows recurrence. Metastases in the regional lymph nodes are extremely rare as are disseminated metastasis [5].

Case presentation

A 52 years old Iranian male was admitted to the Department of Dermatology, Shahid Sadoughi University in Yazd (Iran) in September 2009 for a 2 cm multinodular lesion on his scalp, frontoparietal area with an erythematous and partially eroded surfaces and covered by a serocrust. The lesion was hard and attached to the hypodermis. The patient had history of irradiation to his scalp in adolescence. There was no evidence of lymph node enlargement. The lesion was small exophytic mass, yellowish in colour, and hard in consistency. Its size was about 2 × 1 cm in diameter. No enlargement of cervical lymph nodes was evident. The clinical impression was basal cell carcinoma or metastasis. Chest X-ray, abdominal sonography & CT-scan of head and neck revealed no remarkable findings suggesting visceral carcinoma. Laboratory data including tumor markers were within normal limits. The patients was in good condition and without subjective difficulties (e.g. pain, fever and weight loss). The patient underwent wide surgical excision and the raw area covered by split thickness skin graft. The specimen was fixed in 10% neutral formalin and embedded in paraffin. Five micron sections were stained with hematoxylin and eosin. Histologically, the tumor was located in the dermis and hypodermis and consisted of solid nests and small cords in a dense fibrocollagenous stroma. Solid tumor nests consisted of tumor cells which were basaloid and polymorphic, with hyperchromatic nuclei. A few mitotic figures were observed. A palisading arrangement of tumor cells, keratinous cysts, apocrine and follicular differentiation were not observed. There were no connection between the tumor nests and epidermis. The ex-

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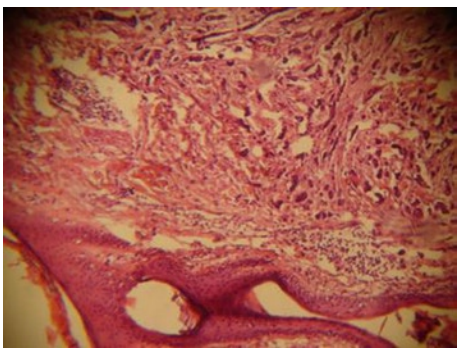

Fig. 1 Section shows skin tissue with ductal structures and small cords of cells in fibrous stroma (HE staining × 10)

tent of tumor invasion could not be determined from the biopsy specimen, because the tumor had spread beyond the edge of excision (Fig. 1). The diagnosis was syringoid eccrine carcinoma.

Discussion

Syringoid eccrine carcinoma (SEC) was first described as eccrine epithelioma (Basal cell carcinoma with eccrine differentiation) by Freedman and Winkelman in 1969, and it is an extremely rare skin appendage tumor [6]. It usually affects subjects in the fourth to seventh decades of life and manifests as a solitary, firm nodule or plaque positioned on the scalp, face, or, more rarely, other sites [7]. Clinically our case mimics the most frequent appearance of SEC: a large sclerotic plaque on the scalp. He is the first case of SEC from Iran. The presented case had history of irradiation to his scalp in adolescence. We did not find any relationship between SEC and previous radiotherapy, although previous irradiation to the head and neck is a predisposing factor to some of head and neck malignancies, such as thyroid carcinoma [8]. According to this point our case is unique. Histologically SEC resembles syringoma by presenting with ductal, cystic and tad poled-shaped structure. SEC differs from syringoma by its cellularity and deep invasiveness. SEC needs to be differentiated from basal cell carcinoma (BCC) microcystic adnexal carcinoma (MAC) primary cutaneous adenoid cystic carcinoma (PCACC) and visceral carcinoma with skin metastasis. Distinction from BCC Should be straight forward in view of the lack of a palisading arrangement of tumor cells InSEC. MAC should be differentiated from SEC as MAC contains foci of eccrine and follicular differentiation and is composed of nests and strands of basaloid cells that form keratin filled cysts whereas SEC does not usually form keratin filled cysts. PCACC usually shows a predominant cribriform pattern of tumor growth and histologic evidence of mucin production, which are lacking in SEC [9]. It may also be difficult to differentiate SEC from metastatic adenocarcinoma. The patient should be checked for visceral carcinoma carefully. The tumor grows slowly but is locally invasive and can metastasize to regional lymph nodes and subsequently to the bones and this has been reported in only one case [10]. Moy *et al* suggested that therapy of SEC is mostly surgical and nowadays Mohs micrographic surgery is the method of choice [11]. Subsequently our patient underwent wide local surgical excision followed by split thickness skin graft. Excellent aesthetic and functional result were obtained and recurrence was not detected after 3 months.

Conclusion

In conclusion, we emphasize that SEC is a rare primary disease. Its clinical appearance is not well characterized. A complete local excision is effective in making both definitive diagnosis and treatment. It is rarely metastasizing to lymph nodes or distant organs and has a good prognosis.

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Squamous cell carcinoma of hypopharynx in a patient with history of celiac disease

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Abstract Celiac disease is a gluten-related malabsorption in small intestine occurring in genetically susceptible patients. In this disease the risk of many malignancies is increased the most important of which being non-Hodgkin lymphoma of small intestine. Other malignancies include adenocarcinoma of small intestine and squamous cell carcinoma of esophagus and melanoma. As to our knowledge so far only one case of celiac disease associated with hypopharyngeal squamous cell carcinoma has been reported. In this article we presented a patient suffering from celiac disease with squamous cell carcinoma of hypopharynx. She underwent chemotherapy and radiation therapy, unfortunately however she died because of progress of disease. So, in patients with celiac disease we should pay attention to various malignancies and when cases of cancers are accompanied by malabsorption we must think of celiac disease involvement.

Keywords celiac disease; malignancies; squamous cell carcinoma; hypopharynx; chemotherapy; radiotherapy

Celiac disease is a chronic inflammatory condition of the small intestine, and a gluten-induced disease in genetically susceptible children and adults and causes nutrient malabsorption, often accompanied with diarrhea and weight loss [1, 2]. Several types of malignancies are associated with this disease [1–8]. The most common is beings malignant lymphoma, 80% occurring in the small intestine [3]. The next most frequently found malignancy was adenocarcinoma of the small intestine, followed by carcinoma of the esophagus [3]. Other malignancies occurring more frequently than expected are carcinoma of the pharynx lung, breast, testis [3] and melanoma [4]. As to our knowledge, so far only one case of hypopharyngeal carcinoma has been reported [5, 6], and in this article we presented the second case.

Case report

A fifty four years old housekeeper lady with a six years history of celiac disease referred to our Department after taking a biopsy from her hypopharyngeal area that revealed squamous cell carcinoma. She complained from dysphagia, hoarseness and a red painful mass in her neck, that was fistulized to her skin. CT scanning showed a large polypoid mass about 6 cm × 4 cm in the left posterolateral aspect of hypopharynx that was extended to pharynx, larynx and parapharyngeal spaces (Fig. 1).

Metastatic work up didn't show any suspicious involvement. The patient received two courses of chemotherapy with cisplatin (100 mg/m^2) and continuous infusion of 5-flurouracil (1000 mg/m^2) for 96 hours. CT scanning and fiberoptic laryngoscopy were repeated, but response was less than 50% and as a rule she would become a candidate of total pharyngolaryngectomy, however, the patient and her family refused the procedure. Since we didn't have any clinical trial for such situation in our country we decided to try radiation therapy and after three steps of shrinkage tumoral area received 7000 cGy with cobalt 60 machine. One month after completion of radiation therapy course CT scanning was repeated and a mild decrease in tumor size was seen and her symptoms were partially relieved. Six months later her symptoms and specially dysphagia and dyspnea were progressed and due to economic situation we decided to try weekly metotheraxate 40 mg/m^2, and she became better for several weeks until the disease was progressed, and then she received paclitaxel 175 mg/m^2, and carboplatin with AUC = 6, but she died due to progressive disease 14 months after diagnosis.

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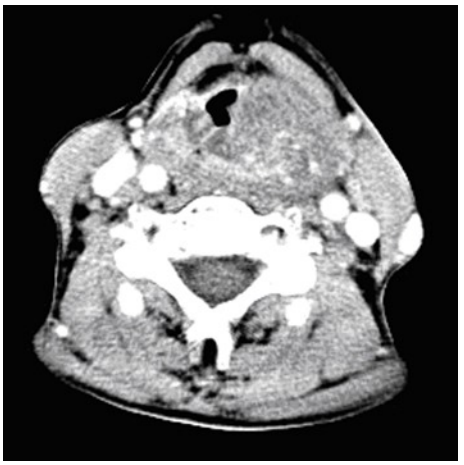

Fig. 1 Neck CT scan before treatment

Discussion

Malignancy is a serious concern in celiac disease [5]. The precise risk of malignancy in adult celiac disease is about 8%–10% [5]. The most common malignancy that associated with celiac is T-cell lymphoma although recently B-cell lymphoma was also reported [2–9]. Incidence of small-bowel adenocarcinoma is increased in celiac disease [3–7, 9]. Some of the studies have shown that there may be an increased risk of esophageal [4–7] and pharyngeal carcinoma [4–6]. In one report only a single terminal hypopharyngeal squamous cell carcinoma was detected in a celiac disease patient with lymphoma [5, 6]. Other malignancies occurring more frequently than expected were carcinoma of the lung, breast, testis and melanoma [3, 4]. Different observations are reported on colorectal cancers, some of them claiming that celiac disease increases the risk of these malignancies, however, others don't show such a situation specially in the untreated patients that may be at a lower risk than general population, because of diarrhea and decrease presence of toxic agents in colon [5, 6].

The mechanism for the development of malignancies in patients with celiac disease is not known. However, increased intestinal permeability of environmental carcinogens, chronic inflammation, chronic antigenic stimulation, the release of pro inflammatory cytokines, immune surveillance problems, and nutritional deficiencies due to the disease or the gluten-free diet have been suggested [4].

A research on 210 patients with celiac disease in England showed that with a gluten-free diet for five years, the risk of developing cancer of patients was not increased when compared with the general population. The risk is increased, however, in those taking a reduced gluten, or a normal gluten, diet with an excess of cancers of the mouth pharynx and esophagus and also of lymphoma [3]. On the other hand, Green *et al* confirmed an increased risk of non-Hodgkin's lymphoma despite a gluten-free diet for a mean of about 5 years [4].

In our case the patient with a known five years history of celiac disease who had a gluten-free diet after diagnosis was studied. Advanced hypopharyngeal cancer was seen. The patient was treated by chemotherapy and radiation therapy with no excessive side effects.

Conclusion

To the best of our knowledge, this is the second case of hypopharyngeal squamous cell carcinoma reported in a patient with a history of celiac disease in whom the disease was seen despite receiving a gluten-free diet for five years, fortunately however the patient could tolerate chemotherapy and radiation therapy although her response was not much significant.

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