

Original Article

Establishment of a Lung Cancer Biobank of a Southern Chinese Population

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ABSTRACT There is an increasing need for establishment of biobanks for human cancer, which may facilitate basic and clinical researches as well as the development of novel approaches to early diagnostics, prevention and treatment, including personalized medicine. Herein we report the establishment of a lung cancer biobanks using biological samples from a lung cancer patient population in Southern China. Since 2007, we have collected lung cancer tissue from 1,054 patients with lung cancer, from whom 11,895 frozen tumor and normal matched tissues, 4,899 tissue paraffin blocks, and 3,562 blood serum samples were accumulated. The information on clinical manifestations, laboratory tests, and follow-up was maintained by an independent information management system, including three data sets. The primary data set included frozen tissue specimens, formalin-fixed paraffin-embedded tissues, blood specimens and clinical and long-term follow-up data. The secondary data set included DNA, RNA and proteins extracted from corresponding tissue specimens. And the tertiary data set contained improved genome, RNA and proteomics correlation analysis with relevant clinical and long-term follow-up data. The lung cancer biobanks is accessible to academic research and public services. To our best knowledge, this biobanks represents the first lung cancer tissue reservoir in China and should facilitate the basic and clinical research of this disease and developing diagnostic markers and novel therapeutic modalities.

KeyWords: lung cancer; biobanks; tissue bank; southern Chinese population

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The incidence of lung cancer in 2007 is estimated to be 213,380 with 160,390 deaths in the United States. It will contribute to 31% of male and 26% of female cancer-related deaths and is the largest cause of cancer-related mortality in both men and women (1). According to histopathologic and biological perspectives, lung cancer is a highly complex neoplasm (2). More than 99% of lung cancers are carcinomas. However, within that broad category exist several histological subtypes, including small cell lung carcinoma (SCLC; 20% of lung cancers) and the non-small cell lung cancer (NSCLC) subtypes squamous cell carcinoma (30%); adenocarcinoma, including the noninvasive subtype bronchioloalveolar carcinoma (40%); and large cell carcinoma (9%) (3). The frequency of lung cancer histological subtypes has shifted in the past decades: adenocarcinoma has surpassed squamous cell carcinoma as the most

common type of lung cancer, and the incidence of SCLC is steadily decreasing.

Advances in understanding the basic science of cancer are dependent upon, and to great extent limited by, the availability of high quality appropriate tissue samples for research. Increasingly, the introduction of new therapeutic agents is also dependent on demonstrated efficacy in human tumour tissue or cell cultures. Recent acceleration in the availability of new technological platforms, dubbed 'the omics revolution', also makes great demands on any tissue available for research at the DNA, mRNA and expressed protein levels (4). Development of a tissue and blood (serum, plasma, and circulating cells) bank with specimens obtained from patients with lung cancer, including detailed clinical data, is of the utmost importance. As we can achieve potential research goals (1): identify molecular tissue and blood (e.g., genetic polymorphism and serum proteomic) markers predictive of survival, recurrence, and metastasis development in patients with lung cancer; (2) establish characteristics of precursor lesions and the field cancerization phenomenon in lung cancer pathogenesis by smoking status, gender, and, very importantly, ethnic background; (3) identify molecular tissue and blood markers to predict response to and survival benefit from radiotherapy, chemotherapeutic or targeted therapeutic agents at time of recurrence (5).

Different responses in the treatment of lung cancer are attributable to racial differences (6). The large geographical span

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between northern and southern China leads to large differences in terms of climate and dietary habits. In the north of China where an arid climate prevails, the diet is meat-based and the northern Chinese population is closer to having an East Asian ethnic origin. Whereas, in the south of China, the climate is humid encouraging more vegetable-based diet, and the population mostly has South Asian ethnic origins. The investigation of the hemorheological reference value in healthy people of different cities of China (Beijing, Qiqihaer, Yantai, Yibin and Guangzhou) indicated that the discrepancy of hemorheological indices was different between northern and southern China (7, 8). On the other hand, in northern China, air pollution caused by burning coal indoors and/or outdoors during the long heating season may play an important role in lung cancer etiology. Coal consumption in northern China is much higher than southern China (9). So the establishment of lung cancer biobanks in southern China will help researchers find features and special therapeutic targets for lung cancer in the southern Chinese population.

Based on these considerations, we tried to establish the Lung Cancer Biobanks at Guangzhou Institute of Respiratory Disease and State Key Laboratory of Respiratory Disease, Guangzhou, PR China. The biological samples such as lung cancer tissues, control tissues, and blood, as well as donor-related clinical data have been collecting from a Southern Chinese Population. The major activities and services include collecting and banking freshly frozen tissue specimens from excess surgical material and biopsies, collecting formalin-fixed paraffin-embedded tissue specimens, processing and banking blood components and maintaining a tissue database with links to clinical and follow-up data. The collections are going on and will last for ten years. The independent information management system is controlled by an independent group. It was hoped that these data can guide the selection of clinical treatment method and can be extremely helpful in judging the prognosis of lung cancer patients.

Materials and methods

Tissue origins

Tissues collected in our lung cancer biobank were mainly from Southern Chinese patients with lung cancer who received treatment in the form of surgery, chemotherapy and radiotherapy in Guangzhou Institute of Respiratory Disease. At the same time, samples from bunamiodyl, inflammatory pseudotumor and pulmonary fibrosis patients are also collected as controls of benign diseases. The collection began in 2007 and mainly includes frozen lung cancer tissue specimens (including paired nonneoplastic tissues), formalin-fixed paraffin-embedded tissue specimens (tissue blocks) and blood specimens. A flowchart for the set-up a lung cancer biobank is outlined in Fig 1.

Collection of frozen tissue specimens

Surgical specimens were immediately snap-frozen in liquid nitrogen in situ after resected in the operating room in order to minimize the action of hypoxic phenomena on genetic expression. One pathologist in operating room decides whether it is possible to separate one or more fragments for freezing without this operation affecting the correct pathological diagnosis of the process (10). Those areas with massive ischemia and/or necrotic phenomena must be avoided. This entire process is carried out in the most aseptic conditions possible. Whenever possible, nonneoplastic tissue of the same organ (adjacent tissue: 2-5 cm to the cancer tissue edge and distant tissue: more than 5 cm to the cancer tissue) is selected for freezing and for fixation and paraffin embedding. Samples are then frozen by direct immersion in liquid nitrogen. Components of such specimens can be preserved well and widely applied in DNA, RNA and protein research, especially available to RNA research demanding strict preservation conditions (11-13).

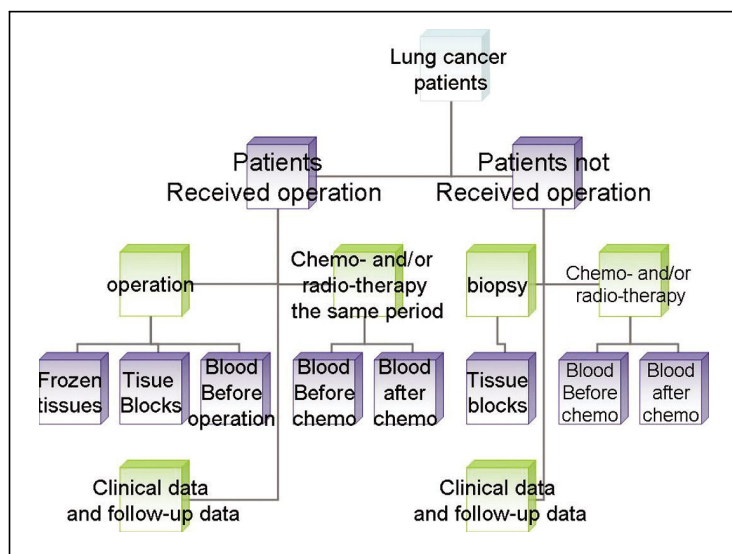


Fig. 1 Tissues were mainly from patients with lung cancer who received treatment in the form of surgery, chemotherapy and radiotherapy. The collection mainly includes frozen lung cancer tissue specimens (including paired nonneoplastic tissues), formalin-fixed paraffin-embedded tissue specimens (tissue blocks) and blood specimens.

Conservation of frozen tissues and security systems

The blocks of frozen neoplastic and non-neoplastic tissue are properly identified so that they are not used for diagnosis, except when this is absolutely necessary. Tissues are stored at temperatures below -196°C in liquid nitrogen tank with the necessary security measures to avoid thawing and/or exposure to excessive changes in temperature. They include: connection to the hospital's emergency power supply, triple alarm system (local visual and acoustic, remote to hospital personnel, telephone to tumor bank members, etc.) and keeping a nearby empty freezer (14-16).

Formalin -fixed paraffin -embedded tissue specimens (tissue blocks)

Formalin-fixed paraffin-embedded tissues are from patients who received operation or biopsy. Corresponding paraffin blocks, glass slides and immunostains are collected and researchers can request unstained sections, H&E-stained sections, or thick slices for RNA/DNA retrieval from paraffin blocks through the biobanks (17).

Collection of blood samples

Blood samples were collected from patients who underwent surgical operations and/or chemotherapies in Guangzhou Institute of Respiratory Disease. Blood from each patient before and after surgery and/or chemotherapy is centrifuged after which separated plasma/serum and blood cells are collected to be preserved in liquid nitrogen. These blood specimens are later provided for disease tracking and observation of drug efficacy (18).

Identification of samples

Samples were organized by designated people and each sample to be processed by the biobanks is immediately identified in accordance with the tissue identification protocol, with clear reference to the tissue type (normal or pathological).

Clinically relevant material and long-term follow-up data

Clinically relevant material and long-term follow-up data collection was carried out by full-time staff. All data were recorded by Hard disk, CD-ROM and paper synchronously (19, 20).

Ethical aspects

All neoplastic tissues accepted by the biobanks in particular biopsies, surgical specimens, and necropsy samples, absolutely respecting the procedures that guarantee correct ethical activity.

Data security

Specific computers were used as databases containing personal information which permits the identification of the patient (name, gender, age, clinical record number, pathology reference number, member of staff in charge of the case, sample number and amount of the samples). The collections and activity of the biobanks are run and controlled by a specific group of people (21-23).

Results

Specimen constituents of the biobanks

The collection is going on and will last for ten years. From January 2007 to May 2009 total cancer cases have reached 1,054 and total sample copies have reached 20,356 (11,895 frozen tissues, 4899 tissue blocks, and 3562 blood specimens).

Constituent ratio of gender

Of the 1054 cancer cases, 821 (77.9%) were from male patients and 233 (22.1%) were from females (Fig 2A). Constituent ratio of gender was 3.52:1 male vs female patients.

Constituent ratio of age

The youngest patient was 14 years old and the oldest one was 89 years old. Age distribution for the lung cancer biobanks showed a normal distribution pattern. Tumor specimens collected from patients aged 41 to 70 years accounted for 80.4% [(223+351+274)/1054=80.4%] of the total number of cases (Fig. 2B).

Constituent ratio of lung cancer and benign lesions

We also found that about 40% of the cases were malignant tumors and 60% were benign lesions based on pathological examination. The constituent ratio of lung cancer to benign lesions was 0.66:1. (Fig. 2C).

Constituent ratio of tumor types

Lung adenocarcinoma and lung squamous cell carcinoma accounted for the majority of the total surgical specimens: adenocarcinoma specimens represented 57.19% of the total number of surgical resection specimens, followed by squamous cell carcinoma, 25.31%. Small cell lung cancer often loses the opportunity to surgery because of early transfer and is often treated by radiotherapy and chemotherapy. Therefore Small cell lung cancer shares a smaller proportion of surgical specimens and mostly just blood specimens are collected for this type of cancer. Large cell carcinoma specimens represent 0.57% of surgical resection specimens. Others included Sarcoma, Carcinosarcoma and Lymphoepithelioma-like carcinoma and cumulatively represent 8.54% of surgical resection specimens (Fig. 2D).

Lung cancer family data

Three lung cancer families were found during the process of collection. Blood specimens have been collected from each lung cancer patient and the immediate family members in the family. At least 1-2 patients in each lung cancer family received surgery in Guangzhou Institute of Respiratory Disease and frozen tissue specimens and formalin-fixed paraffin-embedded tissues were also acquired from them.

Database management

A relatively independent information management system has been set up and controlled by a specific group. Frozen tissue specimens, formalin-fixed paraffin-embedded tissues, blood specimens and clinically relevant material and long-term follow-up data construct the first grade data. Serial detection of DNA, RNA and pro-

tein of the first grade data construct the second grade data. Improved genome, RNA and proteomics correlation analysis with clinically relevant material and long-term follow-up data construct the third grade data. These data can guide the selection of clinical treatment method and can be extremely helpful in judging the prognosis of lung cancer patients.

Service of the lung cancer biobanks

The goal of the lung cancer biobanks was to facilitate biomedical research by using tissues collected from Southern Chinese patient populations. The following services are currently offered to support this goal: (a) Banked frozen tissue specimens: DNA, RNA and protein research group especially available to RNA research demanding strict preservation conditions; (b) Formalin-fixed paraffin-embedded tissue: DNA and morphological study; (c) Banked frozen serum/plasma specimen: DNA, RNA and protein research

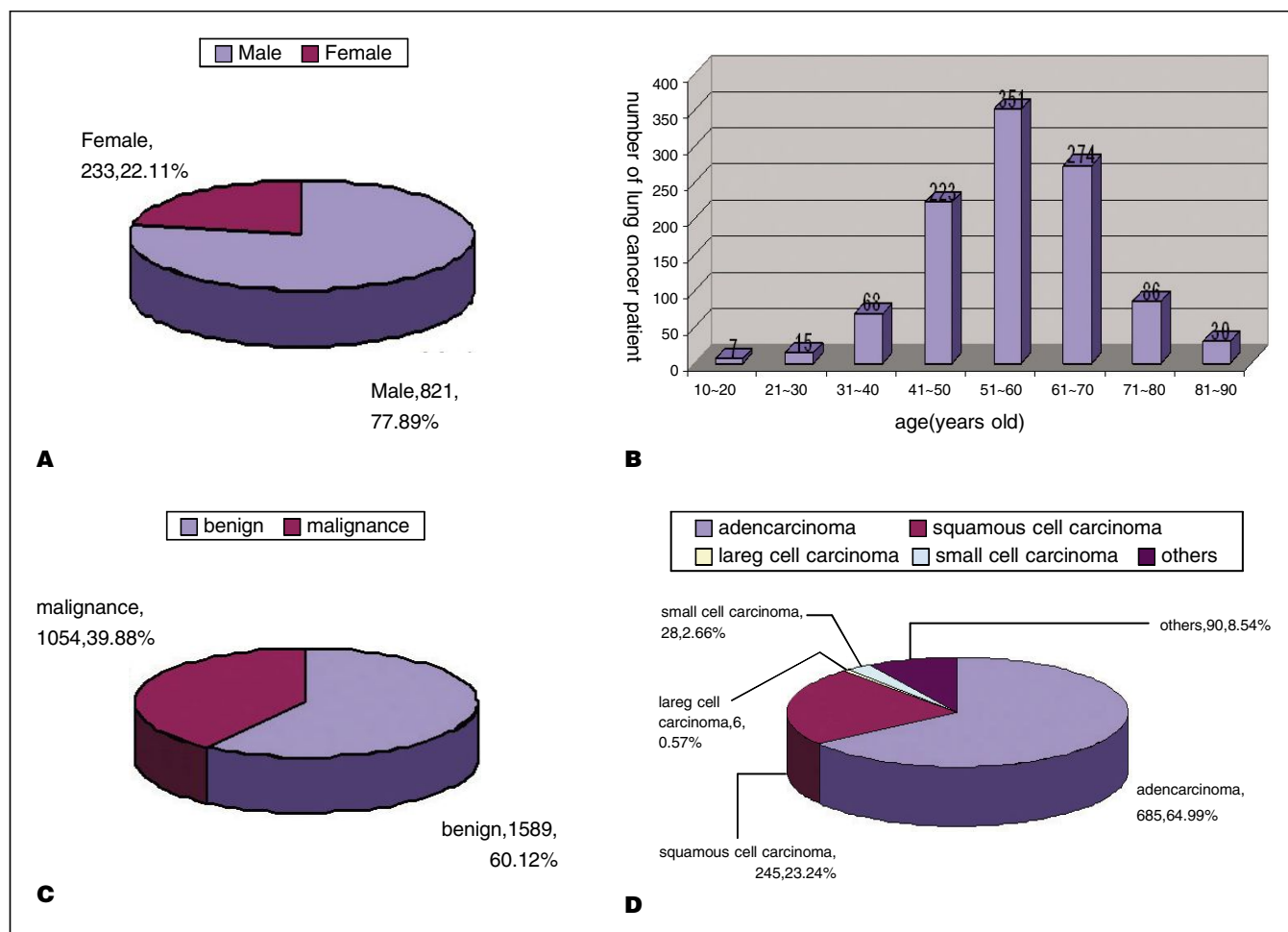


Fig.2 (A) Constituent ratio of gender was 3.52:1 to male vs female patients; (B) The youngest patient was 14 years old and the oldest was 89 years old. Age distribution for the lung cancer biobanks showed a normal distribution pattern. Tumor specimens collected from patients aged 41 to 70 years accounted for 80.4% total case number; (C) About 40% of cases were malignant tumors and 60% cases were benign lesions based on pathological examination and the Constituent ratio of lung cancer to benign lesions was 0.66:1; (D) Lung adenocarcinoma and lung squamous cell carcinoma accounted for the majority of the total surgical specimens: adenocarcinoma specimens representing 57.19% of the total number of surgical resection specimens followed by squamous cell carcinoma, 25.31%. Small cell lung cancer often loses the opportunity to surgery because of early transfer and is often treated by radiotherapy and chemotherapy.

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Discussion

Research biobanks are organizations that collect and store tissue samples for use in scientific research (12, 15). However, there were only formalin-fixed paraffin-embedded tissues in department of pathology, which can't meet the requirement of current research. Most RNA and protein studies require samples to be preserved in profound hypothermia. In some countries such as the United States, Italy, etc., the establishment of cryopreserved tissue sample banks is progressing (12, 15, 16). Establishment of such a bank is useful since ethnic and geographical differences lead to differences in gene expression profiles, protein profiling and response to treatment. As it is well known, Eastern and Western races are correlated with different responses to the treatment of lung cancer in several studies (24).

In order to be able to carry out research in cooperation with other biobanks such as banks in north China and the US, the lung cancer biobanks of Southern China set up by Guangzhou Institute of Respiratory Disease and State Key Laboratory of Respiratory Dis-

ease in accordance with the international standards. Tissues were quick frozen in liquid nitrogen, blood samples were collected during every therapy period (before and after surgery and chemotherapy) and paraffin specimens were documented for indispensable morphological study. In medical tissue archives, samples are usually not directly associated with clinical data, which substantially limits their usability for research. Samples in the tissue bank with corresponding data from medical records greatly enhance their value for scientific use. To solve this problem, our biobanks contain biological samples as tissues and blood, and donor-related clinical data. The advantages of clinically relevant information and long-term follow-up data are clear. By studying samples collected before and after surgery or chemotherapy and with the availability of clinical and follow-up data, we can track the complete course of these treatments and their effects. Also, joint research of different biobanks on patients' treatment response can help identify different therapeutic targets.

Serving its original purpose of providing services for researchers in Oncology, our lung cancer biobank has been available for use in various types of research since its establishment: South Chinese proteomics research; South Chinese micro-RNA research; South Chinese EGFR Mutation and amplification research; Smok-

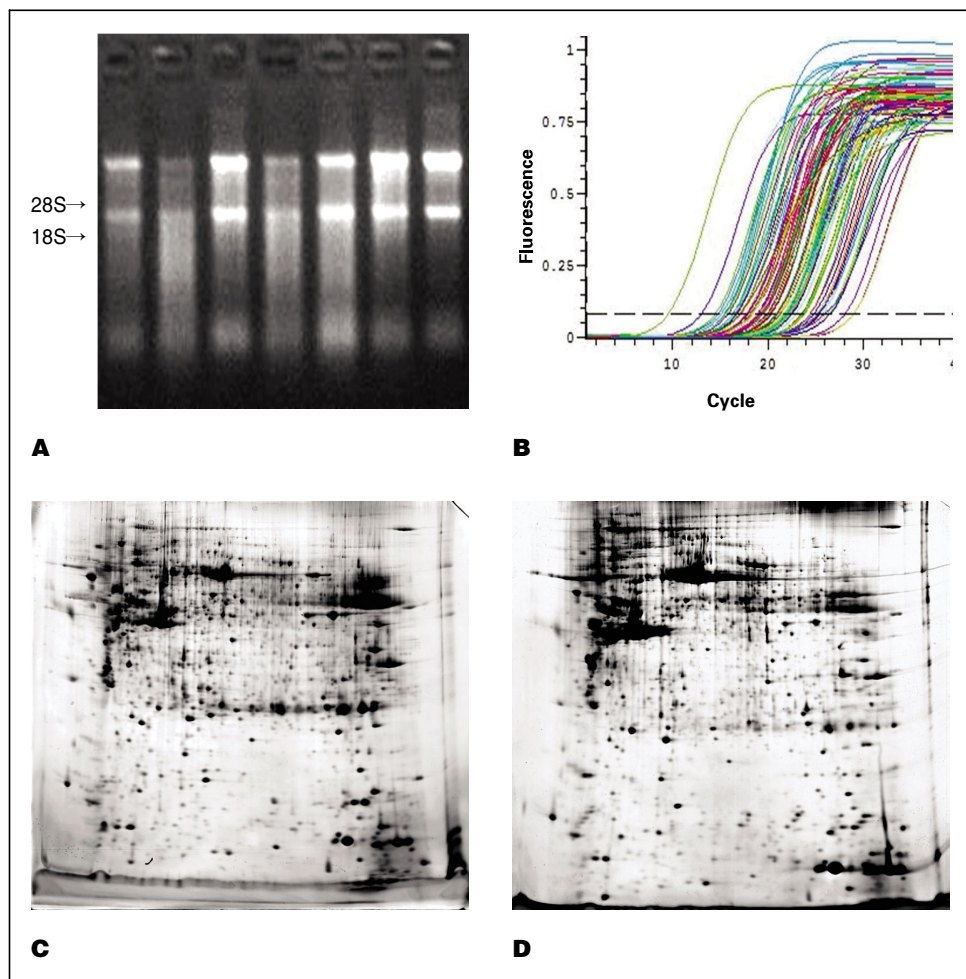


Fig.3 (A) Good-quality RNA by electrophoresis as evidenced by the 18S and 28S bands is seen in all specimens; (B) miRNA expression profiling, qRT-PCR results showed that the quality of preservation of specimens was in line with the requirements of detection of miRNA expression profiling; (C) 17cm-long SDS-PAGE gel two-way electrophoresis of lung adenocarcinoma, with 1312 ± 36 protein spots, the quality of preservation of specimens was in line with the requirements of proteomics experiments; (D) 17cm-long SDS-PAGE gel two-way electrophoresis of adjacent tissues, with 1186 ± 28 protein spots.

ing-related study of Ca²⁺ channel; etc. The quality of preservation of specimens was in line with the requirements of RNA by electrophoresis, detection of miRNA expression profiling, as well as proteomics experiment. (Fig 3)

Collect these samples and clinical and follow-up data just the beginning for our biobanks. In order to provide more service to clinical and basic cancer research of lung cancer, we will continue to expand the resource base of the specimen collection. We will also establish lung cancer biobanks of different regions, different nationalities and different tumor types. With the development of molecular biology, tumor target therapy will make a good progress and more individuals will benefit from it. We also look forward to establishing databases of patients with relapse or of those who failed to respond to treatment, which can provide researchers with new information on treatment and can help explain the mechanisms of drug-resistance.

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References

- Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin* 2007; 57: 43-66.
- Minna JD, Gazdar A. Focus on lung cancer. *Cancer Cell* 2002; 1: 49-52.
- Travis WD, Brambilla E, Muller-Hermelink HK, Harris CC. Tumours of the lung. In: WD Travis, E Brambilla, HK Muller-Hermelink, CC Harris (Eds.), *Pathology and Genetics: Tumours of the Lung, Pleura, Thymus and Heart*. Lyon: International Agency for Research on Cancer (IARC), 2004:9-124.
- Hall GL, Kademani D, Risk JM, Shaw RJ. Tissue banking in head and neck cancer. *Oral Oncol* 2008; 44: 109-15.
- Raso MG, Wistuba II. Molecular pathogenesis of early-stage non-small cell lung cancer and a proposal for tissue banking to facilitate identification of new biomarkers. *J Thorac Oncol* 2007; 2:S128-35.
- Derby KS, Cuthrell K, Caberto C, Carmella SG, Franke AA, Hecht SS, et al. Nicotine metabolism in three ethnic/racial groups with different risks of lung cancer. *Cancer Epidemiol Biomarkers Prev* 2008; 17: 3526-35.
- Sun ZH, Cai ZH, Zhang WY, Xu DX, Zhu AX, Fu YW. The investigation of the hemorheological reference value in healthy people of Guangzhou. *Chin J Hemorrh* 2005; 15: 137-9.
- Lu XM, Guo Y, Xie XL, Dai Q. Normal values of hemorrheologic index in population of Yibin Region. *Chin J Hemorrh* 2004; 14: 379-80.
- Wang QS, Lin XP. Lung cancer epidemiology in mainland China. *Chin J Clin Oncol* 2006; 3: 244-53.
- Nather A, Phillips GO, Morales Pedraza J, Lee CC. Training tissue bank operators: the International Atomic Energy Agency (IAEA)/National University of Singapore (NUS) 10 years of experience. *Cell Tissue Bank* 2009; 10: 183-90.
- Martinez-Pardo ME, Mariano-Magana D. The tissue bank at the Instituto Nacional de Investigaciones Nucleares: ISO 9001:2000 certification of its quality management system. *Cell Tissue Bank* 2007; 8: 221-31.
- Asslaber M, Abuja PM, Stark K, Eder J, Gottweis H, Trauner M, et al. The Genome Austria Tissue Bank (GATiB). *Pathobiology* 2007; 74: 251-8.
- Loken SD, Demetrick DJ. A novel method for freezing and storing research tissue bank specimens. *Hum Pathol* 2005; 36: 977-80.
- van Veen BE. Human tissue bank regulations. *Nat Biotechnol* 2006; 24: 496-8.
- Tettamanti M, Tralamazza S, Berati M, Molteni M, Gamba N. Human research tissue banks: the ATRA Project for establishing a human research tissue bank in switzerland. *Altern Lab Anim* 2005; 33: 29-36.
- Pellegrinelli A, Collini P, Carbone A. Frozen tissue bank: experience of the Tumor National Institute of Milan. *Pathologica* 2005; 97: 193-4.
- Kanzawa F, Sugimoto Y, Minato K, Kasahara K, Bungo M, Nakagawa K, et al. Establishment of a camptothecin analogue (CPT-11)-resistant cell line of human non-small cell lung cancer: characterization and mechanism of resistance. *Cancer Res* 1990; 50: 5919-24.
- Knox K, Kerr DJ. Establishing a national tissue bank for surgically harvested cancer tissue. *Br J Surg* 2004; 91: 134-6.
- Gilbertson JR, Gupta R, Nie Y, Patel AA, Becich MJ. Automated clinical annotation of tissue bank specimens. *Stud Health Technol Inform* 2004; 107: 607-10.
- Casas JM. Human tissue bank for research. *An Sist Sanit Navar* 2004; 27: 7-10.
- Esther Martinez-Pardo M, Lourdes Reyes-Frias M. The tissue bank at the national nuclear research institute in Mexico. *Cell Tissue Bank* 2003; 4: 163-8.
- Collins SM, McHugh K, Croitoru K, Howorth M. The establishment of a national tissue bank for inflammatory bowel disease research in Canada. *Can J Gastroenterol* 2003; 17: 107-9.
- Chu TY, Hwang KS, Yu MH, Lee HS, Lai HC, Liu JY. A research-based tumor tissue bank of gynecologic oncology: characteristics of nucleic acids extracted from normal and tumor tissues from different sites. *Int J Gynecol Cancer* 2002; 12: 171-6.
- Hida T, Ogawa S, Park JC, Park JY, Shimizu J, Horio Y, et al. Gefitinib for the treatment of non-small-cell lung cancer. *Expert Rev Anticancer Ther* 2009; 9: 17-35.