RESEARCH ARTICLE

Luciferase Assay to Screen Tumour-specific Promoters in Lung Cancer

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Abstract

Objective: Specific promoters could improve efficiency and ensure the safety of gene therapy. The aim of our study was to screen examples for lung cancer. Methods: The firefly luciferase gene was used as a reporter, and promoters based on serum markers of lung cancer were cloned. The activity and specificity of seven promoters, comprising CEACAM5 (carcinoembryonic antigen, CEA), GRP (Gastrin-Releasing Peptide), KRT19 (cytokeratin 19, KRT), SFTPB (surfactant protein B, SP-B), SERPINB3 (Squamous Cell Carcinoma Antigen, SCCA), SELP (Selectin P, Granule Membrane Protein 140kDa, Antigen CD62, GMP) and DKK1 (Dickkopf-1) promoters were compared in lung cancer cells to obtain cancer-specific examples with strong activity. Results: The CEACAM5, DKK1, GRP, SELP, KRT19, SERPINB3 and SFTPB promoters were cloned. Furthermore, we successfully constructed recombinant vector pGL-CEACAM5 (DKK1, GRP, SELP, KRT19, SERPINB3 and SFTPB) contained the target gene. After cells were transfected with recombinant plasmids, we found that the order of promoter activity from high to low was SERPINB3, DKK1, SFTPB, KRT19, CEACAM5, SELP and GRP and the order for promoters regarding specificity and high potential were SERPINB3, DKK1, SELP, SFTPB, CEACAM5, KRT19 and GRP. Conclusion: The approach adopted is feasible to screen for new tumour specific promoters with biomarkers. In addition, the screened lung-specific promoters might have potential for use in lung cancer targeted gene therapy research.

Keywords: Luciferase assay - lung cancer - tumor-specific promoter - gene therapy - tumor biomarker

Introduction

Over the past 10 years, lung cancer has become the leading cause of cancer death in worldwide. More than 7,000 people died from lung cancer in Taiwan in 2010 and more than 150,000 people died from lung cancer in US in 2010 according to the new statistics (Jemal et al., 2010; Shieh et al., 2013). Since the overall survival of lung cancer has improved only minimally over the past four decades, novel therapeutic approaches are needed (Siegel et al., 2013).

Suzuki et al. have been found that combination of genomic modification to achieve tumor-selective replication and capsid modification to enhance infectivity yields more potent oncolytic adenoviruses for use in cancer treatment (Suzuki et al., 2001). What’s more, tumor-specific promoters (TSPs) regulated target genes can be specifically expressed in cancer cells. Therefore, TSPs could greatly increase the therapeutic efficacy and security of gene therapy for cancer which is considered a breakthrough in the field.

It has been reported that transfection of NSCLC (non–small cell lung cancer) cells with both human NIS (sodium iodide symporter) and TPO (thyroperoxidase) genes resulted in an increase in radioiodide uptake and retention and enhanced tumor cell apoptosis (Huang et al., 2001). An adenovirus mediated transgene expression system of the hNIS (human sodium iodide symporter) under the control of hTERT (human telomerase reverse transcriptase) promoter has the potential to become an effective wide-spectrum yet highly specific anti-cancer strategy (Shi et al., 2010). The study of Tadashi et al. showed the possibility of gene therapy using the cell type-specific promoter of CEA (carcinoembryonic antigen) gene against CEA-producing adenocarcinoma of the lung and adenovirus carrying the NIS gene linked to the CEA promoter induced high levels of tumor-specific radioiodide accumulation in HCT 116 cells (Osaki T, 1994; Scholz et al., 2005). Survivin promoter is a cancer-specific promoter for various cancers which may be useful controlling gene expression in cancer cells and in cancer gene therapy (Bao et al., 2002; Chen, 2004). Reciprocal binding of

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CTCF (CCCTC binding factor) and BORIS (Brother of the Regulator of Imprinted Sites) to the NY-ESO-1 (a member of the cancer–testis family of antigens) promoter mediates epigenetic regulation of this CT (cancer/testis) gene in lung cancer cells which may be a novel strategy to augment immunogenicity of pulmonary carcinomas (Hong JA, 2005). Protein expression is regulated by genes, and gene transcription and replication are subject to a number of complex regulatory elements (Levine and Tjian, 2003; Kelly and Scarpulla, 2004). Since promoter is the most representative element, screening for specific promoters could improve efficiency and ensure the safety of gene therapy. Therefore, the aim of our study was to screening more specific promoters for lung cancer marker genes.

In this study, six cell lines were transfected by recombinant plasmids which were verified by agarose gel electrophoresis. Furthermore, the activity and specificity of seven promoters, including CEACAM5 (carcinoembryonic antigen, CEA) promoter, GRP (Gastrin-Releasing Peptide) promoter, KRT19 (cytokeratin 19, KRT) promoter, SFTPB (surfactant protein B, SP-B) promoter, SERPINB3 (Squamous Cell Carcinoma Antigen, SCCA) promoter, SELP (Selectin P, Granule Membrane Protein 140kDa, Antigen CD62, GMP) promoter and DKK1 (Dickkopf-1) promoters were compared to obtain lung cancer-specific promoters with strong activity. We anticipate that the results of this study could be benefit for exploring the mechanism and gene therapy for the lung cancer.

Materials and Methods

Cell lines and culture

Human lung cancer cell lines, including A549, H446 and H460, were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in RPMI 1640 containing 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), 100 units/mL of penicillin, 100 μg/ml of streptomycin, and 0.25 μg/ml of amphotericin B and maintained at 37°C in a humidified 5% CO₂ atmosphere. Cells in good condition were seeded in 6-well cell culture plate.

Extraction of DNA genomes and concentration determination

When the cell fusion rate was over 90%, the genome DNA was extracted from each cell line using the Universal Genomic DNA Extraction kit Ver.3.0 (Takara Shuzo Co.) according to the manufacturer’s instructions. Furthermore, the concentrations of extracted DNA genomes were determined by measuring the ultraviolet (UV) absorbance employing a UV visible spectrophotometer (Thermo Scientific, USA).

Candidate promoters cloning and verification

The cutting sites of restriction enzymes (Fermentas) identified by the software of primer 5.0 version (Primer, Canada) were provided in Table 1. The sequences of cloned promoters were verified by gel electrophoresis detection. Briefly, mixtures in centrifuge tubes were centrifuged in a portable centrifuge (microOne Mini Personal Centrifuge, Tokyo) for 5 s. Then, agarose gel electrophoresis detection was conducted on PowerPac Basic electrophoresis apparatus (BIO-RAD) after incubating all the tubes in a water bath for 2 h at 37°C.

Vector plasmids extraction and verification

The domesticated laboratory strain E. coli DH5α were grown in LB (Luria broth) medium supplemented with 40 μg/ml ampicillin (HyClone, Logan, UT) when required for selection of plasmids. Vector plasmids were extracted from E. coli DH5α harboring the pGL3, sAAV-CMV-Luc and pRL-TK plasmids (Promega, USA) by using the Plasmid Mini Preparation Kit (Beyotime, China). Then the pGL3 plasmid vector, pRL-TK co-transfection vectors and sAAV-CB-EGFP plasmids were digested for verification. Briefly, the reactants were blended and centrifuged in a portable centrifuge for 5 s. Next, agarose gel electrophoresis detection was performed on PowerPac Basic electrophoresis apparatus (BIO-RAD) after incubating in a water bath for 2 h at 37°C.

Construction of recombinant plasmids

Restricted DNA products verified by agarose gel electrophoresis and plasmids were recovered using a gel extraction kit (Beyotime, China) according to the manufacturer’s instructions. The mixture system with the volume of 10 μl including 2 μl recovered plasmid (50-100 ng), target gene fragments (molar ratio of linear vector molecule DNA to exogenous DNA molecule was between 1:1 and 1:10) and double- distilled water (ddH₂O) were incubated in a water bath for 6 min at 45°C to melt and reanneal the cohesive ends. And the mixture was rapidly transferred to an ice bath for 3 min. Then, the volume was raised to 20 μl by addition of 1 μl 10× T4DNA ligase Buffer, 0.5 μl of 5 U/μl T4 DNA Ligase (Takara Shuzo Co.), ddH₂O and incubated at 14 - 16°C overnight.

Recombinant plasmids transfection

Plasmids were purified using a QIAGEN Plasmid Midi Kit (Qiagen) and detected by agarose gel electrophoresis. Then, highly purified plasmid DNA was used for cell transfection. The vectors loading firefly luciferase and Renilla luciferase reporter gene were used to transfect lung cancer cells. Renilla luciferase reporter gene and firefly luciferase reporter gene were served as control and experimental reporter gene, respectively. All transfection experiments for cell lines were carried out in triplicate.

Luciferase luminescence detection

Cells were washed twice by 1 × PBS of pH 7.0 after the culture medium was removed. Then, lysates were prepared

| Table 1. The Restriction Enzyme Cutting Sites |
|---------------------------------------------|
| Promoter | Enzymes | Single restriction sites (bp) |
| GRP | TaqI | 533/675 |
| CEA | Smal | 485/430 |
| SP-B | KpnI | 293/775 |
| KRT | Smal | 395/788 |
| CMV | XbaI | 409/182 |
| DKK | Mulu | 882/167 |
| GMP | KpnI | 157/787 |
| SCCA | HindIII | 263/530 |
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Figure 1. Gel Electrophoresis Photograph Showing the Extracted Genome DNA from Each Cell Line. Lane 1: H446 genome DNA; Lane 2: A549 genome DNA; Lane 3: H460 genome DNA; Lane M: DNA marker DL15000

by applying 250 µl of 1 × PLB (Passive Lysis Buffer) and scraping for 15min at room temperature. A 20 µl aliquot of cell lysate was mixed with 50 µl of Luciferase Assay Reagent II (LAR II). Total 50 µl of Stop & Glo Reagent was then added to the luminometer tube to quench the firefly luciferase (fLuc) reaction and simultaneously activate the renilla luciferase (rLuc) reaction. Renilla luciferase activity was measured immediately with a FB12-Berthold luminometer (Berthold Technologies, Bad Wildbad, Germany) interfaced with a computer was used to trace the luminescence emission over a 12 second period (2 s: pre-read delay, 10 s: numerical reading ) in determination. Data are expressed as relative luciferase activity of duplicate experiments and relative luciferase activity was calculated as the ratio of firefly luciferase activity to renilla luciferase activity.

Statistical analysis

All in vitro experiments were done thrice, and the in vitro experimental data were presented as the mean ± SD and analyzed by Student’s t test for statistical significances. A P-value of less than 0.05 was considered statistically significant.

Results

DNA genomes of human lung cancer cell lines

The electrophoresis photograph showed DNA genomes extracted from A459, H460 and H446 were larger than 15000bp which indicated that the extracted DNA genomes were of high quality (Figure 1). The concentrations of genomic DNA extracted from A459, H460 and H446 were 108 µg/ml, 89 µg/ml and 98 µg/ml, respectively.

PCR products of Promoters

The DNA genomes extracted from A549, H446, H460 cells were used as templates, and PCR cloning products of promoters were detected by agarose gel electrophoresis (Figure 2).

For PCR products of promoters, a clear 1200bp band of GRP promoter, a clear 900bp band of CEA promoter, a clear 1100bp band of KRT promoter, a clear 1078bp band of SP-B promoter, a clear 600bp band of CMV promoter, a clear 1000bp band of GMP promoter, a clear 750bp band of SCCA promoter, a clear 1000bp band of DKK promoter could be observed.

Vector plasmids extraction and verification

The obtained competent state of Escherichia coli cells with high activity were used for the subsequent transformation of recombinant plasmids. The agarose gel electrophoresis images of pGL3-Basic Vector and pRL-TK
Vector were shown in Figure 3. The results indicated that the vector plasmids extracted from E. coli DH5α were correct.

Recombinant plasmid

The recombinant plasmid including pGL3-GRP, pGL3-CEACAM5, pGL3-KRT19, pGL3-SFTPB, pGL3-CMV, pGL3-SELP, pGL3-DKK1 and pGL3-SERPINB3 were identified by agarose gel electrophoresis (Figure 4). The images proved that all the plasmids were recombinant plasmids.

The expression activities of candidate promoters in cell lines

The relative luciferase activity of seven candidate promoters in six kinds of transfected cells (A549, H460, H446, 7721, Hela and 293) were shown in Figure 5. The differences in relative luciferase activity among the candidate promoters were observed with the CMV promoter as control. The order about promoter activity from high to low was SERPINB3, DKK1, SFTPB, KRT19, CEACAM5, SELP and GRP. The specificity and high potential of promoters from high to low order were SERPINB3, DKK1, SELP, SFTPB, CEACAM5, KRT19 and GRP. Therefore, the screened new promoters, such as SERPINB3, DKK1, SFTPB and KRT19, have potential application in gene therapy of lung cancer.

Discussion

Appropriate promoter selection allows for improved gene expression in the respiratory system, such as human polyubiquitin C (UbC) promoter which is one of the lung-specific endogenous promoters (Gill et al., 2009). In this study, seven promoters of lung cancer marker genes including SERPINB3, DKK1, SFTPB, KRT19, CEACAM5, SELP and GRP 7 promoters were cloned. The comparison results of activity and specificity of these seven promoters showed that the screened promoters, such as SERPINB3, DKK1, SFTPB and KRT19, have the potential to be used in the gene therapy for lung cancer.

Studies have shown that direct injection of DNA into tumors can lead to transduction of a significant proportion of the tumor cells (Stewart et al., 1992; Plautz et al., 1993) and that use of a tissue-specific promoter can achieve essentially tumor-specific expression of the...
injected gene (Vile and Hart, 1993). The main method of screening a promoter is to clone promoters according to the sequences of promoters searched in the human genome. The promoters of biological serum markers, including alpha-fetoprotein (AFP) and phosphatidylglycerol (PGC-3) in liver cancer, human kallikrein 2 (HK2) and prostate specific antigen (PSA) in prostate cancer, pancreatic cancer germ resistant (POA) and mucin 1 (MUC1) in pancreatic cancer and neuron-specific enolase (NSE) in lung cancer, have been reported (Qiao J, 2002; Greenberg and Lee, 2007; Johnson et al., 2010; Ma et al., 2010; X Cao, 2011). Therefore, it might be a feasible approach to screen new tumor specific promoters by tumor biomarkers.

SERPINB3, a member of the serpin superfamily, are fundamental for the control of proteolysis. Idiopathic pulmonary fibrosis (IPF) lung samples wherein more extensive fibrosis was observed in patients with higher SERPINB3/B4 expression (Calabrese et al., 2008). Furthermore, it has confirmed that SERPINB3 plays a role in inhibiting inflammation and favoring epithelial proliferation with increased TGF-β secretion and thus the likelihood of consequent fibrogenesis (Lunardi et al., 2011). DKK1, a secreted protein, controls Wnt signaling by binding the lung resistance–associated protein coreceptor and sterically blocking Wnt binding to the receptor complex (Bafero et al., 2001; Mao et al., 2001). Serum DKK1 concentrations were significantly higher in patients with lung cancer than in patients with other malignant tumors or benign lung diseases and healthy controls (Sheng et al., 2009).

SFTPB is a small, hydrophobic peptide that plays an important protective role in the lung (Tokieda et al., 1999). A critical level of SFTPB production is needed to prevent lung disease, and individuals with even one SFTPB mutation limiting SFTPB production may be at risk for lung disease if other factors further decrease SFTPB production (Yin et al., 2013; Yin et al., 2013). Even partial deficiency of SFTPB gene may result in susceptibility to lung injury, suggesting that optimal SFTPB levels are necessary for the maintenance of lung function (Clark et al., 1997; Melton et al., 2003). SFTPB are known to be tissue-specific markers expressed in normal and lung adenocarcinoma (Khoor et al., 1997). Of the 134 lung cancer patient blood samples, 84.3% expressed at least one of the three tumor markers, including tumor specific antigen 9 (TSA-9), Keratin 19 (KRT-19), and Pre-progastrin-releasing peptide (Pre-proGRP). A significant correlation was observed between the number of positive markers and disease stage and progression. Positivity of more than one marker predicted a poor response to therapy and short survival time in non-small cell lung cancer patients (Liu et al., 2008). CEACAM5 is carcinoembryonic antigen-related cell adhesion molecule 5, and CEACAM5 showed high expression in lung tumor tissue (Benlloch et al., 2009). D’Cunha et al. have analyzed the expression of CEACAM5 in 232 lymph nodes from 53 patients with stage I non-small cell lung cancer. In this study, 90.5% of primary tumor samples were positive for CEACAM5 mRNA expression, and 57% of the patients had at least one CEACAM5-positive lymph node and would theoretically be upstaged to stage II (D’Cunha et al., 2002).

In our study, promoters corresponding to the tumor markers of lung cancer described above were screened systematically and the sequences of promoters can be stable obtained for further gene targeted therapy after constructed into plasmid vectors. Therefore, screening novel and tumor-specific promoters based on the tumor biomarkers could promote the research development of target gene therapy.

Studies about tumor-specific promoter have opened up a new and effective way for the efficiency and safety issues of cancer gene therapy. However, some limitations of tumor-specific promoter such as transfection efficiency and stable and long-term gene expression are still not resolved (Podolska et al., 2012). What’s more, the potentially risks of tumor-specific promoters need to be further confirmed, and the optimization and modification of the promoters are very complicated with high technical requirements. Therefore, it is worthy for us to screen more significant tumor-specific promoters and explore more effective optimization program, as well as application methods of promoters.

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References

Bafero A, Liu G, Yaniv A, et al (2001). Novel mechanism of Wnt signalling inhibition mediated by Dickkopf-1 interaction with LRP6/Arrow. Nat Cell Biol, 3, 683-6.
Bao R, Connolly DC, Murphy M, et al (2002). Activation of cancer-specific gene expression by the survivin promoter. J Natl Cancer Inst, 94, 522-8.
Benlloch S, Galbis-Caravajal JM, Alenda C, et al (2009). Expression of molecular markers in mediastinal nodes from resected stage I non-small-cell lung cancer (NSCLC): prognostic impact and potential role as markers of occult micrometastases. Ann Oncol, 20, 91-7.
Calabrese F, Lunardi F, Giacomotti C, et al (2008). Overexpression of squamous cell carcinoma antigen in idiopathic pulmonary fibrosis: clinicopathological correlations. Thorax, 63, 795-802.
Chen JS LJ, Shen L, Rau KM (2004). Cancer-specific activation of the survivin promoter and its potential use in gene therapy. Cancer Gene Ther, 11, 740.
Clark JC, Weaver TE, Iwamoto HS, et al (1997). Decreased lung compliance and air trapping in heterogeneous SP-B-deficient mice. Am J Respir Cell Mol Biol, 16, 46-52.
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D’Cunha J, Corfíts AL, Herndon JE, 2nd, et al (2002). Molecular staging of lung cancer: real-time polymerase chain reaction estimation of lymph node micrometastatic tumor cell burden in stage I non-small cell lung cancer—preliminary results of Cancer and Leukemia Group B Trial 9761. J Thorac Cardiovasc Surg, 123, 484-91; discussion 91.

Gill DR, Pringle IA, Hyde SC (2009). Progress and prospects: the design and production of plasmid vectors. Gene Ther, 16, 165-71.

Greenberg AK, Lee MS (2007). Biomarkers for lung cancer: clinical uses. Curr Opin Pulm Med, 13, 249-55.

Hong JA KY, Abdullahv Z, Flanagan PT (2005). Reciprocal binding of CTCF and BORIS to the NY-ESO-1 promoter coincides with derepression of this cancer-testis gene in lung cancer cells. Cancer Res, 65, 7763-74.

Huang M, Batra RK, Kogai T, et al (2001). Ectopic expression of the thyroperoxidase gene augments radiiodide uptake and retention mediated by the sodium iodide symporter in non-small cell lung cancer. Cancer Gene Ther, 8, 612-8.

Jemal A, Siegel R, Xu J, Ward E (2010). Cancer statistics, 2010. CA Cancer J Clin, 60, 277-304.

Johnson NA, Chen BH, Sung SY, et al (2010). A novel targeting modality for renal cell carcinoma: human osteocalcin promoter-mediated gene therapy synergistically induced by vitamin C and vitamin D(3). J Gene Med, 12, 892-903.

Kelly DP, Scarpulla RC (2004). Transcriptional regulatory circuits controlling mitochondrial biogenesis and function. Genes Dev, 18, 357-68.

Khoo A, Whitsett JA, Stahlman MT, Halter SA (1997). Expression of surfactant protein B precursor and surfactant protein B mRNA in adenocarcinoma of the lung. Mod Pathol, 10, 62-7.

Levine M, Tjian R (2003). Transcription regulation and animal diversity. Nature, 424, 147-51.

Liu L, Liao QH, He P, et al (2008). Detection of circulating cancer cells in lung cancer patients with a panel of marker genes. Biochem Biophys Res Commun, 372, 756-60.

Lunardi F, Villano G, Perissinotto E, et al (2011). Overexpression of SERPIN B3 promotes epithelial proliferation and lung fibrosis in mice. Lab Invest, 91, 945-54.

Ma SH, Chen GG, Yip J, Lai PB (2010). Therapeutic effect of alpha-fetoprotein promoter-mediated tBid and chemotherapeutic agents on orthotopic liver tumor in mice. Gene Ther, 17, 905-12.

Mao B, Wu W, Li Y, et al (2001). LDL-receptor-related protein 6 is a receptor for Dickkopf proteins. Nature, 411, 321-5.

Melton KR, Nessim LL, Ikegami M, et al (2003). SP-B deficiency causes respiratory failure in adult mice. Am J Physiol Lung Cell Mol Physiol, 285, L543-9.

Osaki T TY, Tachibana I, Hosoe S (1994). Gene therapy for carcinomembrionc antigen-producing human lung cancer cells by cell type-specific expression of herpes simplex virus thymidine kinase gene. Cancer Res, 54, 5258-61.

Plautz GE, Yang ZY, Wu BY, et al (1993). Immunotherapy of malignancy by in vivo gene transfer into tumors. Proc Natl Acad Sci U S A, 90, 4645-9.

Podolska K, Stachurska A, Hajdukiewicz K, Malecki M (2012). Gene therapy prospects—intransal delivery of therapeutic genes. Adv Clin Exp Med, 21, 525-34.

Qiao J DM, Sauter BV, Huang Y (2002). Tumor-specific transcriptional targeting of suicide gene therapy. Gene Therapy, 9, 168-75.

Scholz IV, Cengic N, Baker CH, et al (2005). Radioidine therapy of colon cancer following tissue-specific sodium iodide symporter gene transfer. Gene Ther, 12, 272-80.

Sheng SL, Huang G, Yu B, Qin WX (2009). Clinical significance and prognostic value of serum Dickkopf-1 concentrations in patients with lung cancer. Clin Chem, 55, 1656-64.

Shi YZ, Zhang J, Liu ZL, et al (2010). Adenovirus-mediated and tumor-specific transgene expression of the sodium-iodide symporter from the human telomerase reverse transcriptase promoter enhances killing of lung cancer cell line in vitro. Chin Med J (Engl), 123, 2070-6.

Shieh JM, Tang YA, Yang TH, et al (2013). Lack of Association of C-Met-N375S Sequence Variant with Lung Cancer Susceptibility and Prognosis. Int J Med Sci, 10, 988-94.

Siegel R, Naishadham D, Jemal A (2013). Cancer statistics, 2013. CA Cancer J Clin, 63, 11-30.

Stewart MJ, Plautz GE, Del Buono L, et al (1992). Gene transfer in vivo with DNA-liposome complexes: safety and acute toxicity in mice. Hum Gene Ther, 3, 267-75.

Suzuki K, Fueyo J, Krasnykh V, et al (2001). A conditionally replicative adenovirus with enhanced infectivity shows improved oncolytic potency. Clin Cancer Res, 7, 120-6.

Tokieda K, Iwamoto HS, Bachurski C, et al (1999). Surfactant protein-B-deficient mice are susceptible to hyperoxic lung injury. Am J Respir Cell Mol Biol, 21, 463-72.

Vile RG, Hart IR (1993). In vitro and in vivo targeting of gene expression to melanoma cells. Cancer Res, 53, 962-7.

Xiao MY, Wei RC, Zeng Y (2011). Cancer targeting Gene-Viro-Therapy of liver carcinoma by dual-regulated oncolytic adenovirus armed with TRAIL gene. Gene Therapy, 18, 765-77.

Yin X, Meng F, Qu W, et al (2013). Clinical, radiological and genetic analysis of a male infant with neonatal respiratory distress syndrome. Exp Ther Med, 5, 1157-60.

Yin X, Meng F, Wang Y, et al (2013). Surfactant protein B deficiency and gene mutations for neonatal respiratory distress syndrome in China Han ethnic population. Int J Clin Exp Pathol, 6, 267-72.