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ONCOLOGY | RESEARCH ARTICLE

Primary lung cancer cell culture from transthoracic needle biopsy samples

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Abstract: Lung cancer is the leading cause of cancer death in the world. The high mortality rate of this pathology is directly related to its late detection, since its symptoms can be masked by other diseases of lower risk. Although in recent years the number of research related to this subject has increased, molecular mechanisms that trigger this disease remains poorly understood. Experimental models are therefore vital for use in research. Immortalized cell lines have inherent limitations. Explanted tumoral cells obtained by transthoracic needle biopsy can be a potential source of primary culture of human lung tumor cells. Tumor specimens from 14 patients suspected of primary or metastatic lung cancer were obtained by CT-guided transthoracic lung biopsy. Solid tumors were mechanically disaggregated under a stereoscope. Cells were cultured in Base C growth media supplemented with 5% fetal bovine serum in 24-well cell culture plates. Primary lung cancer cell culture was successfully cultured from 12 out of 14 patients. Once a confluent monolayer was obtained, cells were enzymatically harvested and passaged to Petri culture dishes. These primary cell cultures were characterized by cytogenetic tests and gene expression analysis of diagnostic markers. These primary cell cultures revealed chromosome rearrangements and changes in their chromosome complement typical of tumoral cells. Additionally, Fluorescence *in situ* hybridization analysis

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Dr Adriana Rojas holds a bachelor's degree in Biology and Chemistry from the Universidad del Tolima with a masters in Sciences – with emphasis in Genetics – at the University of Los Andes, and PhD in Sciences, Mention: Cellular Molecular Biology and Neurosciences at the University of Chile, 2014. Research Experience in Epigenetics and Lung Cancer, epigenetic control of the expression of genes involved in sexual differentiation, Cytogenetics and Transcriptional Regulation. Dr Rojas is working in lung cancer and how this disease is related with mechanisms of epigenetic control that regulate the transcriptional activity of some genes. These epigenetic mechanisms include DNA methylation, covalent histone modification, and the presence of ncRNA. The research reported in this paper will contribute significantly to the study of the molecular and epigenetic mechanisms that are involved with the onset and progress of the disease.

PUBLIC INTEREST STATEMENT

Lung cancer is the leading cause of cancer death in the world. Experimental models are therefore vital for use in research. In this work, we describe our experience in establishing a protocol to culture primary lung cancer cells from samples obtained by percutaneous puncture of Colombian patients. In addition, we characterized these tumor cell lines by studying cytogenetic abnormalities, fluorescence *in situ* hybridization, RNA isolation, and RT-PCR.

demonstrated that three cultures exhibited *EGFR* amplification. Finally, expression profiles of *CK7, NAPSIN A, TTF1*, and *P63* genes allowed in some cases to confirm sample tumor phenotype. These results demonstrate that primary lung cancer cell culture is possible from percutaneous puncture and provides an important biological source to asses and investigate the molecular mechanisms of lung cancer.

Subjects: Bioscience; Biology; Cancer Biology

Keywords: lung cancer; primary cell cultures; non-small cell lung carcinomas; FISH; chromosome

1. Introduction

To approach lung cancer disease burden, it is fundamental to formulate policy guidelines and public health decision-making, based on mortality analysis, cancer incidence, and prevalence. New cancer cases reported per annum (13%) originate in the lung, with 13 million cases/year. Additionally, 85% occur in people over 65 years old and 60% die early due to disease progression (Ferlay et al., 2007; Jemal et al., 2006). For Colombia, the calculated lung cancer incidence rate was higher than that reported worldwide. In fact, 18.2 per 100,000 new cases in men and 10.3 per 100,000 new cases in women during the period between 2007 and 2011. This disease became the first cause of death in men and the fourth in women in this country (Pardo & Cendales, 2015).

Traditionally, decisions about lung cancer therapy have been based on histological considerations. Tumors are classified according to two histological types: small cell lung cancer and non-small cell lung cancer (NSCLC) (World Health Organization, 2004). NSCLC comprises three different subtypes: squamous cell carcinoma, large cell carcinoma, and adenocarcinoma (ADC). The standard platinum-based chemotherapeutic treatment of advanced NSCLC appears to have reached a plateau in terms of efficacy (Schiller, Harrington, & Belani et al., 2002). A promising treatment strategy involves further subdivision of NSCLC into clinically relevant molecular subgroups according to a classification scheme based on the so-called specific conductive mutations. These mutations occur in genes encoding crucial signaling proteins in cell proliferation and survival, thus cancer cells can depend on the presence of these mutations for survival (Fisher et al., 2001; Ib, 2002).

At present, cancer diagnosis requires a significant amount of procedures, such as morphological examination, immunohistochemical (IHC) techniques, cytogenetics, and/or molecular studies. Based on these many procedures, a large quantity of tissue is required for diagnosis.

There are different diagnostic procedures for obtaining lung tissue depending on tumor location. The most commonly used is bronchoscopy with endobronchial or transbronchial biopsies, CT-guided transthoracic lung biopsy, endobronchial ultrasound (EBUS-TBNA), and surgical procedures for diagnostic purposes such as mediastinoscopy, thoracoscopy, and video-assisted thoracic surgery (VATS) (Dresler, 2013). In general, clinical practice guidelines recommend starting the diagnostic approach by using the least invasive procedure with limitation of a smaller histological sample.

In an effort to develop more rational and effective new therapies, alternatives have been sought for lung cancer biology study. A basic approach has been development of lung cancer cell primary culture for chromosomal and genetic characterizations, in addition to immortalized cell line establishment (Dresler, 2013; Oie, Russell, Carney, & Gazdar, 1996; Reen, 2004; Simms, Gazdar, Abrams, & Minna, 1980). Advantage of cell lines includes their widespread availability, homogeneity in terms of genetic, biochemical, and growth characteristics. However, the process of immortalization may generate karyotypic instability and have major effects on cellular differentiation, morphology, or function compared to *in vivo* conditions (Brodli et al., 2010).

Generally, primary lung cancer cell culture retains morphological and biochemical characteristics of the tumors from which they were derived (Gazdar & Oie, 1986). Retention of these properties increases the relevance of using cell cultures as models for human lung cancer study. In recent decades, the overall use of *in vitro* permanent cell lines has led to great achievements, including elucidating the molecular and translational biology of cancer, and establishment of more effective drugs in lung cancer treatment (Gazdar, Gao, & Minna, 2010; Gazdar, Girard, Lockwood, Lam, & Minna, 2010). Currently, more than 9,000 citations, including several important biomedical findings, have resulted from use of these lines (Gazdar et al., 2010).

Techniques for culturing lung tumor cells from invasive procedure samples, such as mediastinoscopy, thoracoscopy, and VATS, have been successfully standardized in the world. To date, similar work has not been reported, pursuing culture method standardization for minimally invasive procedures, such as bronchoscopy with endobronchial or transbronchial biopsies, percutaneous needle biopsy, or EBUS-TBNA, possibly due to technical difficulties experienced when working with such small samples.

In this work, we describe our experience in establishing a protocol to culture primary lung cancer cells from samples obtained by percutaneous puncture of Colombian patients. In addition, we characterized these tumor cell lines by studying cytogenetic abnormalities, fluorescence *in situ* hybridization (FISH), RNA isolation, and RT-PCR. From this perspective, establishment of cell cultures from percutaneous needle biopsies will allow molecular tests and genetic and epigenetic behavior characterization for the Colombian population.

2. Materials and methods

The study was carried out at the Hospital Universitario San Ignacio/Bogotá, Colombia. The study included 14 patients between October and December 2016 with lung lesions suspected of malignancy. Patients underwent a percutaneous lung biopsy indicated by their physician. This research was under the Colombian Ministry of Health guidelines resolution No. 008430, 1993 and approved by Pontificia Universidad Javeriana School of Medicine Ethics Committee. All procedures were carried out after written signed informed consent was obtained from all participants.

2.1. Sample isolation and culture

Lung cancer tissues were obtained by percutaneous pulmonary puncture with an 18-gauge tru-cut needle, performed by the interventional radiologist. The collected sample was a 15–20-mm long and 1 mm-wide tissue cylinder, fractionated into two tubes. A fraction was used for pathological analysis and immunohistochemistry, and the second for cell culture and its characterization. Tissue samples were rinsed twice with 1 ml sterile phosphate-buffered saline. Then, the tissue was immersed in DMEM/F12 culture medium (Reen, 2004), supplemented with 5% fetal bovine serum (Invitrogen Thermo Fisher Scientific - US), and 1% streptomycin/penicillin (Reen, 2004). Under a stereoscope, samples were mechanically fractionated by cutting with scissors or scalpel, and fragments were cultured into two to three wells and incubated at 37°C, 5% CO_2, and 85% humidity. The cells were resuspended in 300 µl C-based medium (Table 1; Reen, 2004). Once a confluent monolayer was obtained, cells were harvested with trypsin/EDTA and seeded into 35-, 60-, and 100-mm culture dishes for serial passages.

2.2. Tumor cell chromosomal analysis

Karyotype analysis: Cells were seeded and cultured until confluence (80%). Colchicine of 9.2 µg/ml was added to cells and incubated for 3 h. Cells were then harvested using 0.05% trypsin/EDTA and resuspended in 0.075 M KCl. After fixation in 3:1 methanol/glacial acetic acid, cell suspensions were dropped onto ice-cold slides. Last, slides were banded by G-banding protocol. A total of 30 metaphases were evaluated.

Table 1. Culture medium for human lung tumor cells

	C-based medium
Basal medium	DMEM/F12
Sodium selenite	30 nM
Ethanolamine	10 µM
Phosphorylethanolamine	10 µM
Sodium pyruvate	0.5 µM
Glutamine	2 mM
Adenine	0.18 mM
HEPES	15 mM
Supplement	
Insulin	5 µg/ml
Transferrin	5 µg/ml
EGF	10 ng/ml
Cholera toxin	10 ng/ml
Hydrocortisone	100 nM
Triiodothyronine	0.1 nM
Bovine hypothalamus extract	15–30 µg/ml
Bovine serum albumin	0.5%
Fetal bovine serum	5%

Modified from Reen (2004).

FISH: The CytoCell hybridization *EGFR* probe hybridization kit was used, which has a chromosome 7 centromere-specific probe (green), and a gene-specific probe for *EGFR* located in region 7p11.2 (red).

EGRF gene amplification analysis was carried out on 200 interphase nuclei. The score of the obtained results was calculated following Varela-García guidelines (Varella-Garcia, 2006).

2.3. Gene expression profiling

To determine *CK7, NAPSIN A, TTF1*, and *P63* expression, real-time RT-PCR assays were performed on lung tissue samples. mRNA was extracted with Trizol (Life Technologies), according to manufacturer's protocol. Equal amounts of each sample (1 µg) were used for reverse transcription. qPCR was performed using Brilliant II SYBR Green QPCR Master MIX (Roche). GAPDH was used as control gene. To perform gene expression change analysis, RNA from a non-tumor lung culture sample was used (N.C.T).

2.4. Statistical analyses

Student's *t*-test was used for mRNA expression analysis. In all figures, error bars represent mean ± S.E.; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

3. Results

The group analyzed included 14 samples, of which 12 were successfully cultured. These 12 samples corresponded to six patients with primary lung cancer. Five out of six were diagnosed with ADC and one squamous cell carcinoma. The remaining six samples corresponded to metastatic lung tumors, including ductal mammary gland ADC, metastatic prostate ADC, endometrial ADC, follicular lymphoma, and atypical leiomyoma. Last, one case corresponded to solitary fibrous tumor (Table 2).

Pathological sample analysis included IHC studies for all cases, summarized in Table 2.

Table 2. Brief clinical patient details, pathology and lung cancer primary cell culture outcome

Case	Gender	Pathology	Vimentin	CK 7	CK 20	TTF-1	Calretinin	CD5	CD10	P63	Outcome of cultures
1	M	Lung adenocarcinoma	−	+	−	+	−	−	−	−	Successful
2	M	Lung adenocarcinoma	−	+	−	+	−	−	−	N A	Successful
3	F	Lung adenocarcinoma	−	+	−	+	−	−	−	−	Successful
4	M	Lung adenocarcinoma	−	+	−	+	−	−	−	−	Successful
5	M	Lung adenocarcinoma	−	+	−	+	−	−	−	+	Successful
6	M	Poorly differentiated squamous cell carcinoma	−	−	−	−	−	−	−	**+**	**Successful**
7	F	Metastatic ductal adenocarcinoma of the mammary gland	−	−	−	−	−	−	−	−	Successful
8	M	Metastatic prostate adenocarcinoma	−	−	−	−	−	−	−	−	Successful
9	M	Metastatic lung follicular Lymphoma	−	−	−	−	−	+	+	−	Successful
10	F	Metastatic endometrial adenocarcinoma	+	+	−	−	−	−	−	−	Successful
11	M	Metastatic atypical leiomyoma	−	−	−	−	−	−	−	−	Successful
12	M	Solitary fibrous tumor	+	−	−	−	−	−	−	−	Successful
13	M	Adenocarcinoma of lung	−	+	−	+	−	−	−	−	Un successful
14	M	Squamos cell lung adenosarcoma	−	−	−	−	−	−	−	−	Un successful

3.1. Morphological aspect of primary cultures of lung cancer

Twelve samples out of 14 cases were successfully isolated and cultured. Cells obtained from patients 13 and 14 failed to survive *in vitro*. After 6 days of isolation, a limited number of adherent cells could be easily recognized. All 12 primary cultures grew as an adherent monolayer to confluence. Cells were generally, large and exhibited a characteristic epithelial morphology. On occasion they were multinucleated or vacuolated (Figure 1). No cell culture proliferated in suspension, as single cells or clusters of isolated cells.

3.2. Lung cancer primary cell cultures karyotypes

Karyotype for 10 primary cultured cancer cells was performed. Metaphase observation was carried out with an optical photomicroscope. A total of 50 metaphases were observed for all analyzed cases. Presence of clonal chromosomal alterations was determined based on any of the following three conditions: (i) When at least two cells had the same structural alteration, (ii) presence of the same additional chromosome, and (iii) when the same chromosome was missing in at least three cells. Chromosomal alterations was established according to the International System for Cytogenetic Nomenclature (Varella-Garcia, 2006). Primary cultures of nine samples (patients 1–7, 9, and 10) had clonal and non-clonal structural chromosome rearrangements. For one collected sample (patient 8), a universal change in chromosome complement was evidenced, specifically gain of chromosome 7 (Table 3). The most relevant findings corresponded to presence of clonal structural alterations for cases 1–7, 9, and 10, regardless of their origin. Only for one of the analyzed samples (case 6) no clonal alteration was detected (Table 3).

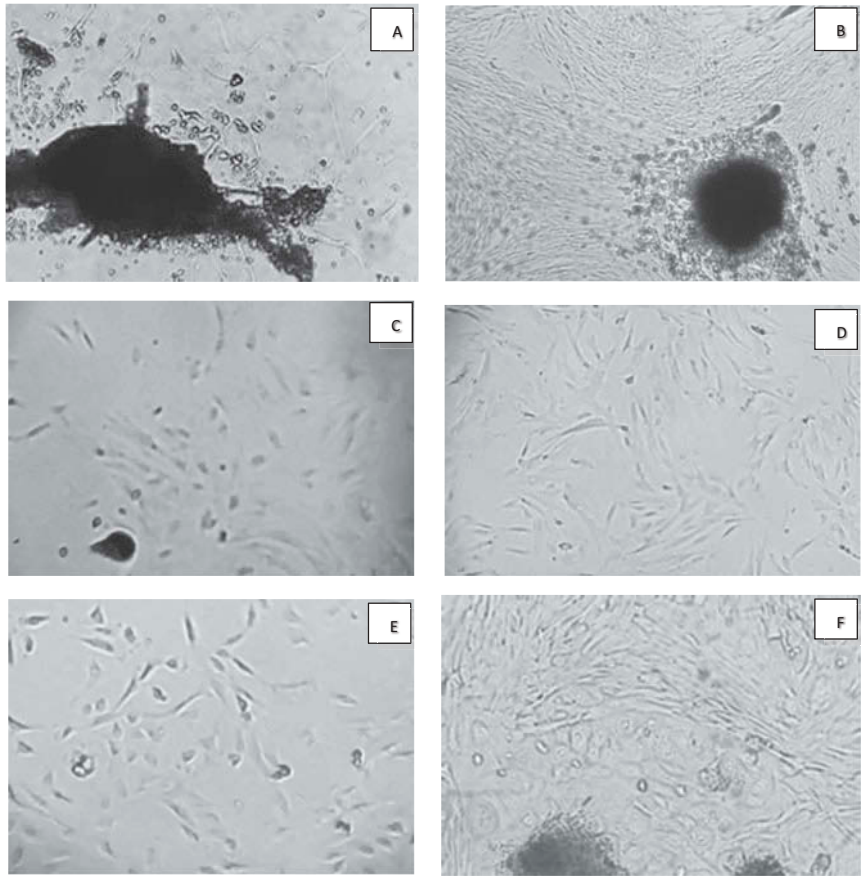

Figure 1. Growth state of cultured cancer cells from lung percutaneous biopses. Cell expansion state of primary cultured lung cancer cells observed under inverted microscope. (a, b): *In vitro* cultured cancer cells derived from lymphoma patient on day 6 and 14, respectively. (c, d): *In vitro* cultured cancer cells derived from adenocarcinoma patients on day 6 and 14, respectively. (e, f): *In vitro* cultured cancer cells derived from squamous carcinoma patients on day 6 and 14, respectively.

Table 3. Results of banding cytogenetic and EGFR FISH analysis

		Chromosomal rearrengements		
Case	**Karyotype/FISH**	**Clonal**	**Non-clonal**	***EGFR* FISH**
1	46,XY,del(Ib, 2002)(q32q34)[2]/184,XXXXYYYY[2]/92,XXYY[6]/46,XY[40] nuc ish(EGFR,D7Z1)x2 ~ 14[32/200]	del(Ib, 2002)(q32q34) Polyploidy	del(Dresler, 2013)(q12q21) del(Oie et al., 1996)(q23) t(12;14)(q24.3;q22) Polyploidy 16n	Positive score = 3.4
2	92,XXYY[2]46,XY[48] nuc ish(EGFR,D7Z1)x2[200]	Polyploidy (4n)	del(Jemal et al., 2006)(q21) add(World Health Organization, 2004)(q13) Trisomy 21	Negative
3	45,X,-Y[40]/90,XX,-Y,-Y[6]/46,XY[4] nuc ish(EGFR,D7Z1)x2[200]	Loss of chromosome Y	r(Y)(p11q12) del(Oie et al., 1996)(p10)	Negative
4	46,XY,del(Varella-Garcia, 2006)(p11)[3]/92,XXYY[8]/46,XY[39] nuc ish(EGFR,D7Z1)x2[200]	del(Varella-Garcia, 2006)(p11)	del(Winokur et al., 2013)(q21) del(Franco & Alberto, 1996)(p10)	Negative
5	44 ~ 46,XY,t(2;7)(p24;q22)[6],amp(Ib, 2002)(?p11.2p13)[2],del(Ib, 2002)(q32)[2],del(Dresler, 2013)(q34)[2][cp10]/92,XXYY[5]/46,XY[15]. nuc ish(EGFR,D7Z1)x3[5/100],(EGFR, D7Z1)x2[95/100]	t(2;7)(p24;q22) amp(Ib, 2002)(?p11.213) del(Ib, 2002)(q32) del(Dresler, 2013)(q34) Polyploidy (4n)	Loss of chromosomes 8, 11, 12, 13, 16, 17, 18, 19, 20, 21, 22, Y	Positive for trisomy
6	46,XY[50] nuc ish(EGFR,D7Z1)x2[200]	None	del(Schiller et al., 2002)(p21) add(Franco & Alberto, 1996)(q13.2) del(Franco & Alberto, 1996)(q13.2)	Negative
7	46,XX,t(1;10)(p13.1;q11.2)[12]/46,XX[38] nuc ish(EGFR,D7Z1)x2[200]	t(1;10)(p13.1;q11.2)	t(X;2)(q27;p21) t(X;2)(q27;p15) t(X;13)(q27;q14) t(1;4)(p36.3;q35) t(1;7)(p21;q11.2) t(3;16)(p14;q24) t(4;5)(q10;q10) t(4;5)(q21;q10) t(4;14)(q21;q13) t(5;14)(p10;q13) del(Ib, 2002)(q22)	Negative
8	47,XY,+ 7[50] nuc ish(EGFG,D7Z1)x3[200]	Trisomy 7	None	Positive for trisomy
9	46,XY,del(Varella-Garcia, 2006)(p12)[4]/46,XY[46] nuc ish(EGFR,D7Z1)x2[200]	del(Varella-Garcia, 2006)(p12)	del(Oie et al., 1996)(q14.2q14.3) del(Varella-Garcia, 2006)(p10) del(Winokur et al., 2013)(p11.2) del(Thomson & Compton, 2011)(q13.1) dmins	Negative
10	46,XX,del(Ferlay et al., 2007)(p10)[2]/46,XX,del(Winokur et al., 2013)(q12.1q12.3)[2]/46,XX[20] nuc ish(EGFR,D7Z1)x2[200]	del(Ferlay et al., 2007)(p10) del(Winokur et al., 2013)(q12.1q12.3)	del(Oie et al., 1996)(q23) Trisomy 18 del(Franco & Alberto, 1996)(p13.3)	Negative

3.3. EGFR amplification status in lung cancer primary cell cultures

We performed the gene amplification of *EGFR* by FISH for all samples. For case 1 (lung ADC primary culture), amplification was detected for *EGFR* with a 3.4 score (frequency 32/200) (Figure 2b). On the contrary, for case 5, FISH analysis revealed an extra *EGFR* gene copy and D7Z1 region signal in 5% of evaluated cells, and for all cells cultured from case 8 (Figure 2d). For this latter one, this finding was compatible with presence of trisomy 7, detected by G-banding analysis (Table 3 and Figure 2c). Last, *EGFR* gene copy number amplification FISH analysis for the remaining cases was negative (Table 3).

3.4. CK7, NAPSIN A, TTF1, and *P63* gene expression profile in lung cancer primary cell cultures

CK7, NAPSIN A, TTF1, and *P63* mRNA expression levels were evaluated in patient's samples. For lung carcinoma of primary origin (1–6), results demonstrated increased *CK7, NAPSIN A* epithelial marker gene expression (Figure 3(a, b)). Samples from lung ADC had increased *TTF1* (1–5) mRNA expression (Figure 3c). Regarding *P63* gene expression analysis, results demonstrated increased expression for cases 2, 4, 5, and 6 (Figure 3d).

In contrast, mRNA expression analysis for samples obtained from metastatic lung cancer results disclosed significantly decreased *CK7, NAPSIN A, TTF1*, and *P63* mRNA expression (Figure 3(a–d)).

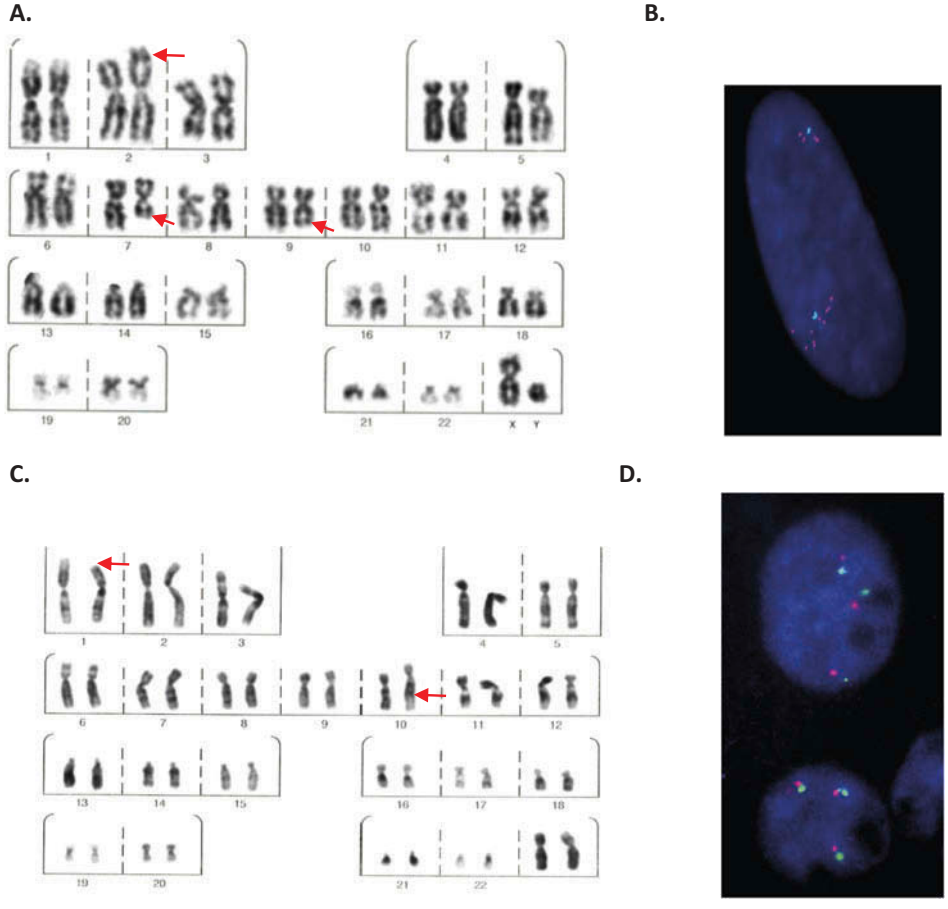

Figure 2. Cytogenetic analysis of cultured cancer cells from lung percutaneous biopsies. (a) Banding karyotype in tumor cells (case 5): 46,XY, t(2;7)(p24; q22), del(Dresler, 2013)(q34). (b) FISH ADENOCARCINOMA, Interphase dual-color FISH of D7Z1probes (centromeric region of 7 chromosome, Green fluorescence), and *EGFR* (*EGFR* locus, red). (c): G banded karyotype of case 1: 1: 46,XX,t (1;10)(p13.1;q11.2). (d) Interphase dual-color FISH of D7Z1probes (centromeric region of 7 chromosome, Green fluorescence) and *EGFR* (*EGFR* locus, red) showing trisomic dosage of chromosome 7 in metastatic prostate adenocarcinoma.

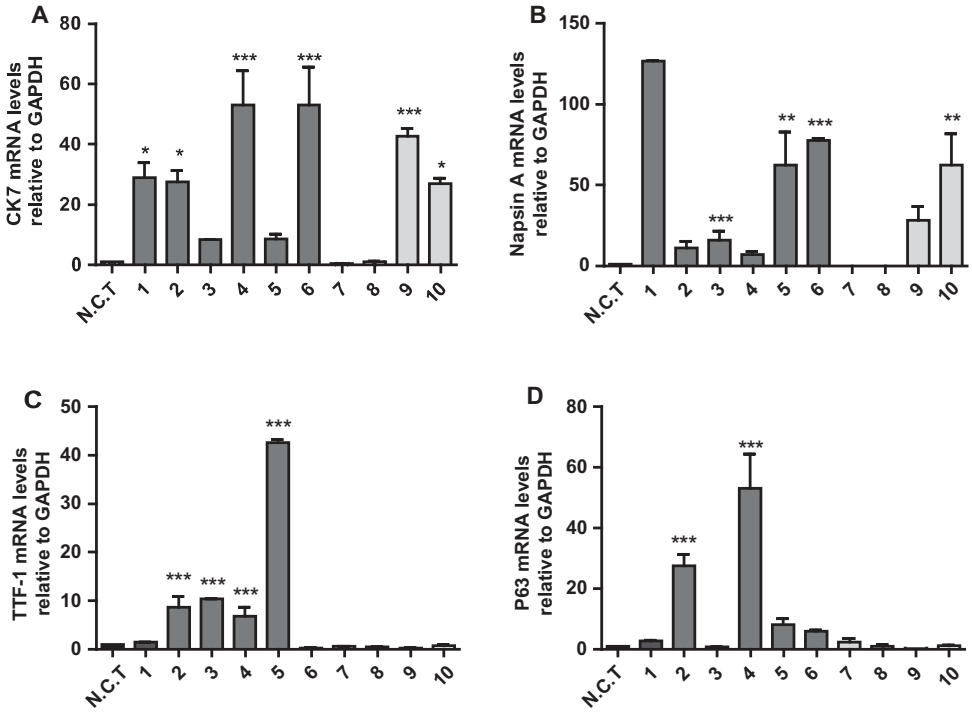

Figure 3. *CK7, NAPSIN A, TTF1,* and *P63* expressions in cultured cancer cells from lung percutaneous biopses. Total mRNA was analyzed for CK7, NAPSIN A, TTF1, and P63 mRNA levels by qRT-PCR using specific primers for these genes. Values were normalized to GAPDH mRNA. Non-tumor lung cell culture sample was used as control (N. C.T). Statistical analyses were performed with respect to pC cells. **, $p < 0.01$; ***, $p < 0.001$; ns: nonstatistically significant differences.

However, it is important to note increased expression of epithelial markers *CK7, NAPSIN A* for cases 9 and 10 were observed (Figure 3(a,b)).

4. Discussion

Lung cancer *in vitro* culture models have limitations, since they favor primary cells to undergo continuous phenotypic changes. Additionally, genetic and molecular alterations may cause changes with time resulting in different characteristics from the tissue of which they were initially isolated. This behavior, typical of established cell lines, prevents the possibility of predicting what actually happens *in vivo*. This, in addition to having a different genetic profile from the original population, is a consideration that justifies establishing new tumor-derived cell lines to pave the way for general understanding of lung cancer, specifically for the Colombian population.

This pilot research aimed at exploring *in vitro* culture conditions and expansion of tumor cells obtained by percutaneous biopsy from patients with suspected lung cancer. Percutaneus biopsy is an indispensable tool in pulmonary abnormality evaluation, because of its high diagnostic accuracy, sensitivity, and specificity for detection of malignancy. Lung needle biopsy plays a critical role to pathologically demonstrate the malignancy, guide cancer stage, and plan treatment (Winokur, Pua, Sullivan, & Madoff, 2013). In the last years, these types of biopsies have been widely used given their high effectiveness in diagnosis. Additionally, it has been shown to be a minimally invasive procedure. Nevertheless, there are no reports in the literature regarding primary cell culture establishment from these types of biopsies.

Fourteen samples were included in this study, where 12 lung cancer cell cultures were successfully established. Cells from two patients under systemic antibiotic treatment failed to achieve *in vitro* cell cultures. It is likely antibiotic treatment could have interfered with cell culture and account for its failure. Therefore, we had an 85% success rate. We have succeeded in consolidating a protocol that clearly describes the methodologies for isolating and expanding tumor cells from percutaneous biopsies of patients with primary or metastatic lung cancer. With some of the cell

cultures reported in this study, our research group has performed transwell invasion assays and silencing assays using lentiviral particles (results not shown), which demonstrate the future utility in the area of investigation.

Conventional cytogenetic sample analysis demonstrated great chromosomic instability, characterized by aneuploidy, polyploidy, deletions, complex translocations, and gene copy number (amplification). Two leading features of cancer cells are abnormal chromosome numbers (aneuploidy and polyploidy) and structural chromosomal rearrangements (Franco & Alberto, 1996). These chromosome aberrations are caused by genomic instabilities inherent to most cancers (Franco & Alberto, 1996). Aneuploidy is caused by underlying erosion of mitotic fidelity called chromosomal instability (CIN), which is defined as a persistently high rate of loss and gain of whole chromosomes (Thomson & Compton, 2011). In general, aneuploidy and CIN are associated with poor prognosis, metastasis, and resistance to chemotherapeutics (Lengauer, Kinzler, & Vogelstein, 2018). Chromosomal rearrangements occur through chromosome structure instability, due to inadequate DNA damage repair (Franco & Alberto, 1996).

Cytogenetic analyzes in the 10 primary cultures herein presented showed different clonal and/or nonclonal chromosomal alterations, whose presence ratified the genetic instability inherent of tumor cells, and allows to reinforce cultured cells tumor phenotype. Primary culture derived from pulmonary ADC (cases 1–5) presented clonal numerical chromosomal anomalies as aneuploidy and polyploidy, which has been reported as characteristic in this type of tumor and is considered a poor prognosis indicator (Fang & Wang, 2014).

In this context, main alterations observed in primary ADCs were in chromosome 7, such as long arm deletions, p11p13 amplification, which houses *EGFR* gene and translocation in mucinous ADC (case 5) t(2;7)(p24;q22). Its rearrangement has not been previously described; therefore, its tumorigenesis is unknown (Scagliotti, Parikh, & Von Pawel et al., 2008). Chromosome 7 houses important genes implicated in tumor development and progress, such as *MET, EGFR, BRAF, EZH2*. All of them are known for their involvement in tumor phenotype alterations. In NSCLC, various gene mutations and amplifications, as well as rearrangements and chromosome copy number alterations (CNAs), have been associated with prognosis and treatment response (Schiller et al., 2002; World Health Organization, 2004).

Other observed clone alterations were t(1;10)(p13.1;q11.2), identified in breast cancer metastatic tumor (case 7), involving fusion of the *TRIM33*/*RET* genes, resulting in aberrant tyrosine kinase activation in thyroid and lung tumors (Heilig, Loffler, & Mahlknecht, 2010). Moreover, 17p12deletion affecting *TP53* gene was present in case 9, which has also been described in different neoplasms, including lymphomas from which this tumor nodule originates (Shaw, Hsu, Awad, & Ja, 2013).

Case 8 corresponded to a metastatic prostate ADC, presenting trisomy 7, which has been previously described in the literature for this class of carcinoma (Cuneo & Castoldi, 2001; Zitzelsberger et al., 1994). It has been proposed presence of chromosomal numerical changes may favor increased mutated gene expression promoting tumor phenotype (Alcaraz et al., 2004). FISH detected the presence of three copies of *EGFR* and D7Z1, thus confirming trisomy 7.

Epidermal growth factor receptor is a transmembrane glycoprotein, with an extracellular epidermal growth factor binding domain and an intracellular tyrosine kinase domain that regulates signaling pathways, controlling cell proliferation (Bethune, Bethune, Ridgway, & Xu, 2010). Epidermal growth factor receptor ligand binding results in autophosphorylation by intrinsic tyrosine/kinase activity, triggering several signal transduction cascades. Constitutive or sustained activation of these sequences of downstream targets is thought to result in more aggressive tumor phenotypes (Bethune et al., 2010). Mutations in epidermal growth factor receptor have

been associated with some lung cancers. Lung ADCs with mutated epidermal growth factor receptor have significant responses to tyrosine kinase inhibitors (Bethune et al., 2010).

In this study, we carried out FISH to detect *EGFR* gene copy number aberrations. As has been consistently reported for lung ADC studies, we observed *EGFR* gene amplification in cultured cells of this carcinoma (case 1). In NSCLC, *EGFR* gene amplification is involved in tumor development and progression, and its presence has been associated with high rates of response to treatment and decreased rate of progression (Gordon, Resio, & Pellman, 2012). For the case of metastatic origin, as previously described, our results demonstrated *EGFR* amplification for case 8 (metastatic prostate cancer). This amplification is the result of the presence of trisomy 7.

Tumor markers include a variety of molecules, such as cell surface antigens, cytoplasmic proteins, enzymes, hormones, oncofetal antigens, receptors, oncogenes, and their products [4]. In this work, we analyzed expression profiles of *CK7, NAPSIN A, TTF1*, and *P63* genes to molecularly characterize primary cultures expanded in our laboratory. Tumoral tissue gene expression has allowed to consider them as onset and progress biomarkers.

CK7 and *NAPSIN A* genes were used as epithelial cell markers (Jagirdar, 2007; Takamochi et al., 2016). Use of immunohistochemistry as a diagnostic tool allowed to establish a comprehensive classification panel with keratins of different molecular weights (CAM5.2, CK903, CK5/6, CK7, CK20) that aid in carcinoma subclassification. For instance, prostate ADC expresses low-molecular-weight keratins (CAM5.2) and is negative for CK7 and CK20 (Jagirdar, 2007; Takamochi et al., 2016). Pulmonary ADC, breast carcinomas, and most female genital tract ADCs express CK7 and are negative for CK20 (Jagirdar, 2007; Takamochi et al., 2016). *CK7* and *NAPSIN A* mRNA expression profiles revealed significant increases in patients with primary lung cancer (cases 1–6), in addition to two patients with metastatic cancer of different origin (cases 9 and 10). It is important to note case 10 corresponded to endometrium metastatic cancer, explaining its epithelial nature.

Other useful high specificity lung cancer markers are thyroid transcription factor 1 (*TTF1*) and *P63*. Expressions of *TTF1* have been established in 80% cases of pulmonary ADC (Carzoglio, Luberti, & El, 2007; Jagirdar, 2007). On the other hand, *P63* is considered to be a good marker for cases of poorly differentiated pulmonary squamous cell carcinoma (Nakamura, Miyagi, Murata, Kawoi, & Katoh, 2002). Even though an elevated level of tumor marker is suggestive of cancer, this alone is not enough to diagnose it. Therefore, analysis of several tumor markers simultaneously constitutes a better diagnostic tool.

Regarding *TTF1* and *P63* expression analysis, we observed an increase in *TTF1* expression for five primary lung cancer samples (cases 1–5), corresponding to IHC findings, thus in agreement with the clinician diagnosis.

TTF1, also referred to as *NKX2-1*, is located on chromosome band 14q13.3. TTF1 is a 38-kDa nuclear protein expressed in the thyroid, lungs, and hypothalamus. TTF1 plays a role in normal lung development and function (Kimura et al., 1996; Minoo, Su, Drum, Bringas, & Kimura, 1999; Yoshimura et al., 2017). TTF1 is also expressed in most lung ADC cases and approximately 80% of small cell lung cancer cases (Misch et al., 2015; Yoshimura et al., 2017; Zhang et al., 2015). *TTF1* functions as a lineage-survival oncogene in lung cancer (Watanabe et al., 2013; Yoshimura et al., 2017). In contrast, *TTF1* also behaves as a tumor suppressor in NSCLC (Yoshimura et al., 2017). Differences in TTF1 protein expression and TTF1 CNAs have been suggested to account for different TTF1 prognostic roles in NSCLC (Yoshimura et al., 2017).

On the other hand, for case 6 with squamous cell lung carcinoma, diagnosis expression profiles demonstrated increased *NAPSIN A, CK7*, and *P63* mRNA expression, and a significant decrease in *TTF1* expression. This joint biomarker analysis allows to confirm the patient's IHC diagnosis. *P63* is expressed in basal cells of stratified squamous and glandular epithelia. In the lung, it has been

mainly studied in different histologic subtypes of epithelial neoplasms, with the highest expression consistently noted in squamous cell carcinomas (Sinna, Ezzat, & Sherif, 2013). Expression frequency in pulmonary ADCs is lower, with most cases showing only focal staining (Sheikh, Fuhrer, & Cieply, 2004; Sinna et al., 2013). Different P63 isoforms are thought to have diverse functions as well. The truncated forms are thought to inhibit cell cycle arrest and driven by transactivating P63/P53 interaction (Yang et al., 1998). Truncated isoforms are preferentially expressed in the basal cell compartment of normal epithelium, and transactivating forms are more widely distributed in benign and neoplastic epithelia (Signoretti et al., 2000). Consequently, different P63 isoforms appear to play a role in maintaining epithelial stem cell population, spurring epithelial differentiation, and inducing neoplasia (Nylander et al., 2002). In the literature, P63 sensitivity and specificity for pulmonary squamous cell carcinoma detection is an effective immunomarker (Uke et al., 2009; Kim and Seon Kwon, 2010).

To conclude, our effort to establish primary cultures of tumor cells obtained from lung by percutaneous biopsy of Colombian patients with suspected primary or metastatic cancer was successful. Additionally, it was possible to perform a characterization of tumor cells by cytogenetic and FISH tests, and analysis of expression profiles of tumor biomarkers. This is the initial approach to our long-term goal of establishing immortalized lung cancer cell lines from Colombian patients that will allow the study of tumor biology in our population and impact current treatments.

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Declaration of interests
The authors report no conflicts of interest.

Author contributions
A.P.R designed the study. A.M.H., L.R., M.J.F., J.A.M., O.M.M., A.P.R. collected the data. L.R., M.J.F., A.C., O.M.M; A.P.R analyzed the data. M.A.M and B.H provided technical and conceptual advice and analyzed the results. M.J.F., A.C., O.M.M, A.P.R wrote the manuscript.

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