Mouse mammary tumor virus-like gene sequences are present in lung patient specimens

Laura M Trejo-Avila1*, Pablo Zapata-Benavides1, Raúl Barrera-Rodríguez2, Isaías Badillo-Almaráz3, Santiago Saavedra-Alonso1, Diana E Zamora-Avila1, Karla Morán-Santibañez1, Jorge A Garza-Sáenz1, Reyes Tamez-Guerra1 and Cristina Rodríguez-Padilla1

Abstract

Background: Previous studies have reported on the presence of Murine Mammary Tumor Virus (MMTV)-like gene sequences in human cancer tissue specimens. Here, we search for MMTV-like gene sequences in lung diseases including carcinomas specimens from a Mexican population. This study was based on our previous study reporting that the INER51 lung cancer cell line, from a pleural effusion of a Mexican patient, contains MMTV-like env gene sequences.

Results: The MMTV-like env gene sequences have been detected in three out of 18 specimens studied, by PCR using a specific set of MMTV-like primers. The three identified MMTV-like gene sequences, which were assigned as INER6, HZ101, and HZ14, were 99%, 98%, and 97% homologous, respectively, as compared to GenBank sequence accession number AY161347. The INER6 and HZ-101 samples were isolated from lung cancer specimens, and the HZ-14 was isolated from an acute inflammatory lung infiltrate sample. Two of the env sequences exhibited disruption of the reading frame due to mutations.

Conclusion: In summary, we identified the presence of MMTV-like gene sequences in 2 out of 11 (18%) of the lung carcinomas and 1 out of 7 (14%) of acute inflammatory lung infiltrate specimens studied of a Mexican Population.

Keywords: MMTV, lung cancer, Mexico

Background

Lung cancer is the most common type of cancer worldwide; it has the highest prevalence and mortality rates in Mexico, and the death rate is increasing [1,2]. There are several risk factors for developing lung cancer; however, smoking is the major risk factor. In countries with a high prevalence of smoking, approximately 90% of the lung cancer diagnoses are attributable to cigarette smoking [3]. Other cases are attributable to occupational exposure to lung carcinogens, such as arsenic, asbestos, beryllium, cadmium, chromium, diesel fumes, nickel, and silica[4-7]. Recently, a viral etiology was proposed, because sequences for gene viral products were detected in patients with lung cancer[8]. Previous studies have reported that human Papillomavirus (HPV) infection may be related to pulmonary adenocarcinoma tumorigenesis[9]. The predominant genotype identified was HPV 16, followed by HPV 18, [10,11] and it has been reported that HPV 16/18 infection is associated with non-smoking Taiwanese female lung cancer[11]. Other studies reported the detection of have detected Epstein-Barr virus in adenocarcinomas and Squamous cell lung cancer[12,13]. Moreover, zoonotic viruses, such as Jaagsiekte sheep retrovirus, have been identified in sheep breeders who develop lung cancer[14].

Recently, Murine Mammary Tumor Virus (MMTV)-like env gene sequences have been identified in humans and are associated with breast carcinoma [15-18]. The whole proviral structure, which shares 95% homology with MMTV, was identified in two human breast cancers and was designated as Human Mammary Tumor
Virus (HMTV) [18]. An epidemiological study on a United States population identified MMTV-like gene sequences in 38% of breast cancer tissue specimens, as compared to <2% in normal breast tissue specimens [16]. The prevalence of MMTV-like gene sequences is: 38% in North America; [16,17] 38% in Italy; [19,20] 38% in Australia; [21] 31% in Argentina; [17] 74% in Tunisia; [20] 16.8% in China; [22] and 4.2% in Mexico [23]. Our group previously identified MMTV-like gene sequences in the INER51 lung cancer cell line, suggesting that these sequences may exist in other tumor types [23]. Here, we extend the search for MMTV-like gene sequences in a Mexican population diagnosed with lung cancer and acute inflammatory lung infiltrate.

Results

Amplification of MMTV env sequences from INERS1
First, we analyzed the DNA from the INER51 lung cancer cell line using primers 1-3 and 5L - 3N. We re-amplified the DNA using primers 2N - 3L and 2N - 3N, as shown in Figure 1. All PCR assays yielded bands with the expected product size, except primers 1-3. This primer set amplified two products: one was 665-bp (expected product), and the other was 500-bp. INER51 was used as a positive control for all subsequent assays.

Amplification of env, LTR, and gag MMTV-like sequences from lung cancer samples
We analyzed 11 lung cancer samples and seven specimens with other lung pathologies (Table 1). The lung samples were amplified with primers 1-3, and nested PCR was performed using primers 5L-3L. In both PCR tests, the sequences were amplified in three samples, as shown in figure 2. To confirm the presence of the MMTV-like gene sequences in the lung samples, the three positive samples were amplified using primers of a LTR-gag region as shown in figure 3.

To discard any possible contamination in the amplifications, PCR reactions were run with reagents and laboratory facilities where they never have worked with cell culture or with MMTV (Laboratory of Genetics, Facultad de Medicina Veterinaria y Zootecnia UANL) performing here the whole process of samples processing.

Sequencing was performed to confirm the identities of the MMTV-like sequences of the PCR products using the 5L-3L primers. The reported nucleotide sequence

| Table 1 Pathology of lung tissues samples included in the study |
|---------------------|---------------------|---------------------|
| Sample | Type | Diagnosis |
| HZ 27 | Biopsy | Mediastinal lymphoma |
| HZ 28 | Biopsy | Infiltrating adenocarcinoma |
| HZ 40 | Biopsy | Infiltrating adenocarcinoma |
| HZ 101 | Bronchial washing | Bronchogenic carcinoma |
| HZ 106 | Bronchial washing | Bronchogenic carcinoma |
| IN 03 | Bronchial washing | Adenocarcinoma |
| IN 06 | Bronchial washing | Adenocarcinoma |
| IN 09 | Bronchial washing | Adenocarcinoma |
| IN 11 | Bronchial washing | Micropapillary adenocarcinoma |
| IN 12 | Bronchial washing | Micropapillary adenocarcinoma |
| IN 14 | Bronchial washing | Lung cancer pulmonary anthracosis |
| HZ 05 | Biopsy | Acute pulmonary inflammatory infiltrate |
| HZ 10 | Biopsy | Acute pulmonary inflammatory infiltrate |
| HZ 14 | Biopsy | Acute pulmonary inflammatory infiltrate |
| HZ 16 | Biopsy | Acute pulmonary inflammatory infiltrate |
| HZ 17 | Biopsy | Acute pulmonary inflammatory infiltrate |
| HZ 32 | Biopsy | Acute pulmonary inflammatory infiltrate |
| HZ 42 | Biopsy | Acute pulmonary inflammatory infiltrate |

PCR was performed using primers 5L-3L. In both PCR tests, the sequences were amplified in three samples, as shown in figure 2. To confirm the presence of the MMTV-like gene sequences in the lung samples, the three positive samples were amplified using primers of a LTR-gag region as shown in figure 3.
In this paper, we report the presence of MMTV-like gene sequences in 2 lung carcinomas and a acute inflammatory lung infiltrate samples that were positive for MMTV when analyzed using the 1-3 and 5L-3L primers. We amplified the MMTV-like LTR-gag region using the primers reported by Liu et al to confirmed their positivity [18] Furthermore, we used the INER51 cell line as a positive control. In 2010, Johal H et al. [25] reported on the detection of MMTV-like env sequences in ovarian, prostate, endometrial, and skin cancers, but not in lung cancer, indicating that MMTV-like presence is not restricted to breast cancer cells. We detected MMTV-like gene env sequences in the INER51 lung cancer cell line [23]. Here, we show that MMTV-like gene sequences exist in lung samples from a Mexican population and support that the presence of MMTV-like sequences is not restricted to breast cancer cells.

A very important aspect to consider is that samples processing an PCR reactions were made also in Laboratory of Genetics of Facultad de Medicina Veterinaria y Zootecnia UANL, where people have never worked with cell lines (including INER51) or MMTV genetic material and therefore the risk of DNA contamination is null.

In this study, we analyzed the MMTV-env sequences in two lung cancer samples and the results suggested that nonsense mutations were caused by deamination (TGG to TGA or TGG to TAG). Human APOBEC3G (APOBEC-related cytidine deaminase, hA3G) deaminates cytosine residues within single-stranded DNA during reverse transcription, resulting in high levels of plus-strand G-to-A mutations [26]. Therefore, hA3G can introduce nonsense mutations, such as TAG or TGA, in the plus-strand coding sequence, since TGG is a target of hA3G. Consistent with this finding, it was reported that most nonsense mutations in the HTLV-1 proviruses in cases of adult T-cell leukemia were caused by deamination [27].

Conclusions

In this study, we detected MMTV-like env gene sequences in three out of 18 lung tissues specimens obtained from Mexican patients. Two samples assigned as INER6 and HZ-101 were isolated from lung cancer specimens, and the HZ-14 sample was isolated from an acute inflammatory lung infiltrate sample. The three identified MMTV-like gene sequences were 99%, 98%, and 97% homologous, respectively, as compared to Gen Bank accession number AY161347. Two of the env sequences exhibited disruption of the reading frame suggesting that nonsense mutations were caused by deamination (TGG to TGA or TGG to TAG).
Methods

Cell Line and Tissue Samples

Eleven lung cancer samples and seven samples of other lung pathologies, including pulmonary anthracosis and acute pulmonary inflammation infiltrate (Table 1), were obtained from the Hospital Regional de Zacatecas in Mexico City and the Instituto Nacional de Enfermedades Respiratorias (INER) in Mexico City. The non-

Figure 4 MMTV-like env gene sequencing. Comparison of env gene sequences amplified from INER6 (GU252129), HZ-101 (HM63470), HZ-14 (HM63471), and INER51 (DQ367729).
small cell lung cancer cell line INER51 was established and obtained from the Instituto Nacional de Enfermedades Respiratorias-SSA, Mexico City, from the pleural effusion of a patient diagnosed with primary lung [28]. This cell line was maintained in DMEM/F-12 with 10% fetal bovine serum (FBS) in (5% CO2) at 37°C.

DNA Isolation
DNA was extracted from tumor samples and cell lines using DNAzol® genomic DNA isolation reagent (Molecular Research Center, Inc., Cincinnati, OH) following the manufacturer instructions. The DNA concentration was determined by measuring the 260/280 nm absorbance of each sample with a Pharmacia Biotech Ultrospec 3000 (Manufacturer name and address).

Detection of MMTV-like gene sequences by PCR
PCR was performed using three set of primers to amplify different specific segments of the MMTV env gene. The 1-3 primers (5′-CCTCACTGCCAGATC-3′, 5′-ATCTGTGGCATACCT-3′) amplify a 665-bp segment and 2N-3N primers (5′-CCTACATCTGCC TGTGTATC-3′, 5′-ATCTGTGGCATACCTAAGG-3′) amplify a 254-bp segment. The LTR3 primers (5′-CGGATGCCTTTAAGAAGG-3′, 5′-GACAGCTTTACAGGTAGCAG-3′) were used to amplify a 595-bp fragment. To amplify a 1,338-bp segment of MMTV LTR-gag, we used the LTR5-GAG 3 primers (5′-GGTGGCAACCAGGACTAT-3′, 5′-GACAGCTTTGCTAACCTTGTG-3′) and the LTR5-LTR3' primers (5′-GGTGGCAACCAGGACTTAT-3′, CGAACAGACAAACACAGC-3′) to amplify a 630-bp segment. The env set primers were described by Wang et al. in 1995 [15]. The LTR5, LTR3, and GAG primers were described by Liu B et al. [18] PCR was performed in triplicate as previously described using standard PCR procedures to avoid contamination. DNA quality was assessed by amplifying a 452-bp fragment of the G3PDH gene using the following primers: forward 5′-ACCCACAGTCCATCGCATCAC-3′ and reverse 5′-TCCACACCCTGTTGGCTGA-3′. The amplified products were analyzed by electrophoresis on a 1% agarose gel. Images were acquired, and analyses were performed using the gel imaging and analysis system (D&RI Ind. Ltd. Transilluminator and Gel-Pro Imager).

DNA sequencing
The MTTV PCR products were ligated into pCR 4-TOPO (Invitrogen, Carlsbad, CA) and transfected into E. Coli (Top10 cells; Invitrogen). The cultures were grown overnight at 37°C in an LB agar plate. Positive colonies were selected and grown in LB broth. Isolation and purification of plasmids were performed with the Rapid Plasmid Purification Systems (Marligen Bioscience, Inc., Ijamsville, MD). To detect the cloned inserts, we performed an EcoRI digestion followed by 1.2% agarose electrophoresis. The positive sequences were analyzed and compared with previously reported MMTV-like sequences (AY161347) using BLAST [29].

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Author details
1. Departamento de Microbiologia e Imunologia, Facultad de Ciencias Biologicas, Universidad Autónoma de Nuevo León (UANL). Ave. Universidad S/N. Ciudad Universitaria, San Nicolás de los Garza, Nuevo León, 66451, Mexico. 2. Departamento de Bioquímica. Instituto Nacional de Enfermedades Respiratorias-SSA México. Calzada de Tlalpan No.4502. Col. Sección XVI, D.F. 14080, Mexico. 3. Hospital Regional de Zacatecas. Guerrerro 116, Col. Centro, Zacatecas, Zacatecas, 98000, Mexico.

Authors’ contributions
Conceived and designed the experiments: TAL, ZBP. Performed experiments: MSK, GSJ, SAS. Analyzed the data: TAL, ZBP, SAS, CRP, TGR. Participated in the specimen and data collection and testing BRR, BAI. Wrote the paper: ZBP, ZAD, TAL. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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References
1. Medina-Moraes F, Salazar-Flores M, García-Sanchez M, Franco-Molina F: Epidemiología descriptiva del cáncer pulmonar en el instituto nacional de enfermedades respiratorias. Rev Inst Natl Enf Resp Mex 2002, 15:149-152.
2. Minna JD, Roth JA, Gazdar AF: Focus on lung cancer. Cancer Cell 2002, 1:49-52.
3. Patro R, Lopez AD, Brencham J, Thun M, Heath C: Mortality from smoking in developed countries 1950-2000. Indirect estimates from national vital statistics. Am J Epidemiol 1994, 143:529-530.
4. Driscoll T, Nelson DI, Steenland K, Leigh J, Concha-Barrientos M, Fingerhut M, Pruss-Ustun A: The global burden of disease due to occupational carcinogens. Am J Ind Med 2005, 48:419-431.
5. Concha-Barrientos M, Nelson DI, Fingerhut M, Driscoll T, Leigh J: The global burden due to occupational injury. Am J Ind Med 2005, 48:470-481.
6. Nelson DI, Concha-Barrientos M, Driscoll T, Steenland K, Fingerhut M, Punnett L, Pruss-Ustun A, Leigh J, Corvalan C: The global burden of selected occupational diseases and injury risks: Methodology and summary. Am J Ind Med 2005, 48:400-418.
7. Consolini D, De Matteis S, Lubin JH, Wacholder S, Tucker M, Pesatori AC, Caporaso NE, Bertazzi PA, Landi MT: Lung cancer and occupation in a population-based case-control study. Am J Epidemiol 2009, 171:323-333.
8. Bouchet L, Valmary S, Dahan M, Didier A, Galateau-Salle F, Brousset P, Degano B: Detection of oncogenic virus genomes and gene products in lung carcinoma. Br J Cancer 2005, 92:743-746.
9. Li Y, Tsai YC, Chen YC, Christiani DC: Human papillomavirus and female lung adenocarcinoma. Semin Oncol 2009, 36:542-552.
10. Syrjanen KJ: HPV infections and lung cancer. J Clin Pathol 2002, 55:885-891.
11. Cheng YW, Chou HL, Sheu GT, Hsieh LL, Chen JT, Chen CY, Su JM, Lee H: The association of human papillomavirus 16/18 infection with lung cancer among nonsmoking taiwanese women. Cancer Res 2001, 61:2799-2803.
12. Kaiari K, Sato Y, Kameya T, Inoue H, Yoshimura H, Ion S, Kikuchi K: Incidence of latent infection of Epstein-Barr virus in lung cancers—an
13. Chen FF, Yan JJ, Lai WW, Jin YT, Su U: Epstein-barr virus-associated nonsmall cell lung carcinoma: Undifferentiated "Lymphoepithelioma-like" Carcinoma as a distinct entity with better prognosis. Cancer 1998, 82:2334-2342.

14. Rocca S, Sanna MP, Leoni A, Cossu A, Lissia A, Tanda F, Satta MP, Palmieri G: Presence of jaagsiekte sheep retrovirus in tissue sections from human bronchioloalveolar carcinoma depends on patients' geographical origin. Hum Pathol 2008, 39:303-304.

15. Wang Y, Holland JF, Blewess II, Melana S, Liu X, Pelisson I, Cantarella A, Stellrecht K, Mani S, Pogo BG: Detection of mammary tumor virus env gene-like sequences in human breast cancer. Cancer Res 1995, 55:5173-5179.

16. Wang Y, Pelisson I, Melana SM, Go V, Holland JF, Pogo BG: MMTV-like env gene sequences in human breast cancer. Arch Virol 2001, 146:171-180.

17. Melana SM, Holland JF, Pogo BG: Search for mouse mammary tumor virus-like env sequences in cancer and normal breast from the same individuals. Clin Cancer Res 2001, 7:283-284.

18. Liu B, Wang Y, Melana SM, Pelisson I, Najfeld V, Holland JF, Pogo BG: Identification of a proviral structure in human breast cancer. Cancer Res 2001, 61:1754-1759.

19. Bindra A, Muradrasoli S, Kisekka R, Nordgren H, Warnberg F, Blomberg J: Search for DNA of exogenous mouse mammary tumor virus-related virus in human breast cancer samples. J Gen Virol 2007, 88:1806-1809.

20. Levine PH, Pogo BG, Kluo A, Coronel S, Woodson K, Melana SM, Mourali N, Holland JF: Increasing evidence for a human breast carcinoma virus with geographic differences. Cancer 2004, 101:721-726.

21. Ford CE, Faedo M, Crouch R, Lawson JS, Rawlinson WD: Progression from normal breast pathology to breast cancer is associated with increasing prevalence of mouse mammary tumor virus-like sequences in men and women. Cancer Res 2004, 64:4755-4759.

22. Luo T, Wu XT, Zhang MM, Qian K: Study of mouse mammary tumor virus-like gene sequences expressing in breast tumors of chinese women. Sichuan Do Xue Xue Bao Yi Xue Ban 2006, 37:844-846, 851.

23. Zapata-Benavides P, Salceda-Akono S, Zamora-Avila D, Vargas-Rodarte C, Barrera-Rodriguez R, Salinas-Silva J, Rodriguez-Padilla C, Tamez-Guerra R, Trejo-Avila L: Mouse mammary tumor virus-like gene sequences in breast cancer samples of mexican women. Intervirology 2007, 50:402-407.

24. Melana SM, Picconi MA, Rossi C, Mural J, Alonio LV, Teyssie A, Holland JF, Pogo BG: Detection of murine mammary tumor virus (MMTV) env gene-like sequences in breast cancer from argentine patients. Medicina (B Aires) 2002, 62:323-327.

25. Johal H, Faedo M, Faltas J, Lau A, Mousina R, Gozzi P, Defazio A, Rawlinson WD: DNA of mouse mammary tumor virus-like virus is present in human tumors influenced by hormones. J Med Virol 2010, 82:1044-1050.

26. Chiu YL, Greene WC: The APOBEC3 cytidine deaminases: aninnate defensive network opposing exogenous retroviruses and endogenous retroelements. Annu Rev Immunol 2008, 26:317-353.

27. Fan J, Ma G, Nosaka K, Tanabe J, Satou Y, Koito A, Wain-Hobson S, Vartanian JP, MatsuoKA: APOBEC3G generates nonsense mutations in human T-cell Leukemia virus type 1 proviral genomes in vivo. J Virol 2010, 84:7278-7287.

28. De Lucia B, Manuel V, Barrera-Rodriguez R: Characterization of human NSCLC cell line with innate etoposide- resistance mediated by cytoplasmic localization of topoisomerase II. Cancer Sci 2005, 96:774-783.

29. Altichieri SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997, 25:3389-3402.