Microsatellite Instability in Chicken Lymphoma Induced by Gallid Herpesvirus 2

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Abstract

Microsatellite instability (MSI) has been found in a range of human tumors, and little is known of the links between MSI and herpesvirus. In order to investigate the relationship between MSI and Gallid herpesvirus 2 (GaHV-2)-induced lymphoma, fifteen Marek’s disease (MD) lymphomas were analyzed through using 46 microsatellite markers, which were amplified by PCR from DNA specimens of lymphoma and normal muscular tissues from the same chicken. PCR products were evaluated by denaturing polyacrylamide gel electrophoresis for MSI analysis. MSI was proved in all lymphomas, at least in one locus. Thirty of the 46 microsatellite markers had microsatellite alterations. These results suggested that GaHV-2-induced lymphoma in chickens is related to MSI, and this is the first report to demonstrate that MSI is associated with the GaHV-2 induced lymphoma in chicken.

Introduction

Herpesviruses are important pathogens associated with a wide range of disease in human beings and animals, and some of them are associated with cancer in their natural hosts. Epstein-Barr virus (EBV) is the etiological agent of nasopharyngeal carcinoma (NPC), African Burkitt’s lymphoma, posttransplant lymphomas (PTLD), Hodgkin’s disease, and some gastric cancers [1,2]. Kapoسي’s sarcoma-associated herpesvirus (KSHV) is linked to Kapoسي sarcoma (KS) and two lymphoproliferative diseases, i.e., primary effusion lymphoma and multicentric Castleman’s disease [1,2]. Marek’s disease (MD), which is caused by Gallid herpesvirus 2 (GaHV-2), is characterized by visceral T-cell lymphomas, paralysis, blindness, and neurological dysfunction in chicken [3]. Apart from an economically important disease affecting poultry health and welfare, MD is a good model for studying the pathogenesis and immune control of herpesvirus-induced oncogenicity [4,5]. Several MDV-encoded genes related to oncogenesis have been identified, such as major oncogene *meq* (Marek’s EcoR I-Q) and viral telomerase RNA [6]. MEQ is considered to be the major viral oncoprotein of MDV and can induce transcriptional activation or repression depending on its dimerization partner and DNA binding specificity. The host response to MDV infection has been analyzed by proteomic and transcriptomic approaches. Differentially expressed proteins were mainly associated with tumor biology, protein folding, signal transduction, immunology, cell proliferation and apoptosis [7]. Studying host responses to pathogens on the gene or protein level has contributed to our understanding of various host-pathogen interactions.

Microsatellite instability (MSI) is defined as a change of any length caused by either the insertion or deletion of repeating units, in a microsatellite within a tumor when compared to normal tissue [8]. It has been variously reported in a range of human tumor types, including lung [9], bladder [10], ovarian [11], colorectum [8], and breast [12] and it is the hallmark of mismatch repair (MMR) system deficiency. Loss of MMR may contribute to tumorigenesis by elevating both the rate of mutations and mitotic recombination.

The information available regarding the relationships between herpesvirus and MSI in carcinogenesis is controversial. Chang et al found that EBV-positive cases in gastric carcinomas showed no MSI positivity [13,14]. However, Wu et al and Leung et al found MSI positivity in both EBV-negative and EBV-positive gastric carcinomas [15,16]. In order to evaluate the involvement of GaHV-2 and MSI in Marek’s disease lymphoma, forty-six microsatellite markers, which showed a high frequency of MSI in primary chicken embryo fibroblasts infected with GaHV-2 in our previous research, were selected from 304 markers to evaluate the frequency of MSI.

Materials and Methods

Experimental Animals and Infective Virus Strain

Specific pathogen free (SPF) White Leghorn Chickens (Merial Vital Laboratory Animal Technology Co., Ltd, China) were kept at the animal isolation facility at Nannong Hi-Tech Co., Ltd (Nanjing, China). A virulent strain of MDV (GaHV-2) J-1 at passage 32, kindly provided by Merial Animal Health Co., Ltd (Shanghai, China), was used to infect the chickens.
Experimental Design

After 21 days post-hatch, forty SPF White Leghorn chickens were intraabdominally inoculated with 1,000 plaque-forming units of MDV J-1 and housed in the isolation facility in separate rooms. All birds were evaluated daily for symptoms of MD, and were examined for gross MD lesions. Chickens which present ataxia or paralysis and moribund state were euthanized by CO2. The experiment was terminated 58 days after infection. All animal experiments were carried out in dedicated negative pressure rooms and conducted strictly in accordance with the laboratory animal guidelines. The protocol was approved by Laboratory Animal Management Committee of Jiangsu Province.

Histopathological Examination

Tissues samples were removed and collected from chicken euthanized during the experiment period and termination, including the liver, spleen, kidney, heart, lung, peripheral nerves, skin, gonads, thymus and bursa of fabricius. Different tissue samples were fixed in 10% formalin and embedded in paraffin wax via a routine process. All sections were stained with hematoxylin and eosin (H&E) and histopathologically examined through using an optical microscope.

Detection of Microsatellite Instability

Microsatellite instability was detected through using gel-based nonradioactive methods described by Shang et al. [17].

Sample collection and genomic DNA extraction. Tumor specimens that were verified visually and histopathologically were collected for MSI analysis. The genomic DNA of tumors and normal muscular tissue samples from the same chicken were extracted with TIANamp Genomic DNA kit (TIANGEN, China), according to the manufacturer’s instructions. Their concentrations were determined by using a BioPhotometer plus (Eppendorf, Germany).

Polymerase chain reaction for amplification of microsatellite markers. Forty-six microsatellite markers (Table 1), which showed a high frequency of MSI in primary chicken embryo fibroblasts infected with GaHV-2 in our previous research [18], were selected from 304 markers to evaluate the frequency of MSI. PCR reaction mixtures contained the following components: 1 μl genomic DNA template (50 ng/μl), 1 μl of Taq DNA polymerase (5 U/μl), 5 μl of 10× PCR buffer, 1 μl of each primer (10 μmol/L), 4 μl of dNTPs (2.5 mmol/L), and 37 μl water. Touchdown PCR amplification was performed in a PTC-200 (Bio-Rad, USA). The initial touchdown cycle comprised denaturation at 96°C for 30 s, annealing at 65°C for 30 s, and extension at 72°C for 30 s. During the touchdown phase, the annealing temperature was decreased at the rate of 1°C for every cycle of the amplification reaction. After 10 touchdown cycles, 25 standard PCR cycles were performed under the following conditions: 96°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, and final extension at 72°C for an additional 5 mins. The PCR reaction was terminated by adding 20 μl of gel loading dye (98% formamide, 10 mmol/L EDTA, and 0.05% bromophenol blue).

Microsatellite instability analysis. PCR products were denatured at 95°C for 3 mins with gel loading dye, and put immediately on ice for 5 mins prior to loading. About 3 μl of the PCR products were loaded onto a 12% denaturing polyacrylamide gel. The gels were run on the DCode universal mutation detection system (Bio-Rad, USA) in 1×TBE at 45°C at 180V. After electrophoresis, the gels were stained with AgNO₃ according to the method of Sanguinetti et al [19].

Results

Symptoms after Inoculation

Clinical signs of infection by GaHV-2 appeared among the treated chickens, including loss of appetite, depression, pallor, and paralysis of the wings, legs and necks. Death occurred in the second week after post-infection, and reached a peak during the 4th and 5th week. Morbidity was 75%, and mortality was 73.3% when the experiment was terminated. Pathological examination of the chickens tissues revealed that fifteen chickens (15/40, 37.5%) had significantly widespread, diffuse lymphomas involving the liver (n = 9), gonads (n = 12), spleen (n = 9), kidneys (n = 15), proventriculus (n = 6) and heart (n = 9). Tumors were found in twelve chickens after death or euthanasia and in three chickens after euthanasia at the predetermined time point. Fifteen kidney tumors were used to microsatellite instability analysis.

Microsatellite Instability Screening

Alterations of microsatellite markers in the GaHV-2-infected samples were identified as differences in the electrophoretic migration or loss of major band(s) in comparison with normal muscular tissue samples DNA (Fig. 1). MSI was detected at one or more loci in all the tumor's analysis (15/15, Table 2). Chicken no. 24 had nine loci (9/30, 30%) displaying MSI. Only one MSI locus was found in chicken nos. 5, 21, and 39.

The frequency of MSI for each marker is shown in Table 3. Microsatellite alterations exist in 30 markers among 46 microsatellite markers. In the tumor samples, the marker that showed the highest frequency of instability (5/15, 33%) was ABR0352. Microsatellite instability was displayed at ABR0086 and MCW0145 in four tumors (27%), and at ABR0007, ABR0387, and ABR0399 in three tumors (20%).

Discussion

Since the initial description of MSI in HNPCC in 1993, MSI has been identified in a wide variety of human cancers, both familial and sporadic [20]. Microsatellite instability (MSI) is a form of genomic instability. Higher MSI frequency is a prominent genetic feature in many tumors. However, there is no study of MSI in Marek's Disease lymphoma induced by GaHV-2. In this study, we collected MD lymphoma tissue specimens, and evaluated MSI frequencies through using 46 microsatellite markers. All lymphoma showed microsatellite instability in at least one locus. These results indicated that MSI was present in Marek’s disease virus-induced lymphoma. Among three serotypes, only serotype 1 MDV (GaHV-2) causes lymphoma formation in chickens, the other two serotypes (GaHV-3 and HVT) are non-pathogenic. MD vaccine strain CVI988 or HVT is effective in the prevention of tumor development but not infection. The MDV oncoprotein Meq differs between oncogenic and vaccine strains [21]. It remains to be seen whether MSI frequencies is different in all kinds of MDV strains.

MSI has been thought to be closely related to mutation of proteins involved in the MMR system, which normally maintains a low rate of spontaneous mutations and corrects replication errors. Many studies noted the phenomenon of mutation of mismatch repair genes in the development and progression of human tumors [22,23]. Lu et al had found that hypothetical protein [mismatch repair ATPase, MutS family] persistently up-regulated in the bursa of fabricius of chickens infected with the highly virulent strain [21]. Interaction of MDV-encoded proteins and host cell pathways will mediate cell proliferation and apoptosis. The transcriptional regulator MEQ is considered to
Table 1. Microsatellite markers used in this study.

| Marker name | Chromosome | Primer (5'-3') | Size (bp) | Repeat array |
|-------------|------------|----------------|-----------|--------------|
| MCW0248     | 1          | GTTGTCAAAAGAAGATGATG | 220–225   | (CA) 9       |
|             |            | TTGCTTTACTGCCCTTTC |          |             |
| ABR0352     | 1          | AAACCTCGGCCAGCCTGCTC | 334       | (CA) 9       |
|             |            | GAAATTAACACCGCGCCACCACG |       |             |
| ABR0329     | 1          | TTTCCAGAGTCACTCATCTC | 314       | (TA) 17      |
|             |            | TCTATGAGTATCTTTCTTC |          |             |
| ABR0522     | 1          | GAATTAGGGAGCTTTGCC | 193       | (CA) 9       |
|             |            | CTTTTGTGATTTGCCCGGT |          |             |
| MCW0145     | 1          | ACTTTATTTCCCAATTTGCT | 212       | (CAA) 6 (CA) 20 |
|             |            | AAACACAATGGCCACCCGAAAC |       |             |
| LEI0146     | 1          | TCAAGGCACCAAGTCTTG | 276       | (AC) 22      |
|             |            | GATCCTTGCCTCTAGATCAT |          |             |
| ABR0204     | 1          | TAATAAAGGTTGGGTGGG | 280       | (CA) 20      |
|             |            | CAGATGTTAAATATGTTGGG |          |             |
| ABR0007     | 1          | ACACCATCCATTGAACAC | 115       | (CA) 11      |
|             |            | ACAAATATGACATTAACGC |          |             |
| MCW0115     | 1          | ATACCAACATCTGCTCTGA | 252       | (CA) 18      |
|             |            | GCATTGTCCTGACTATCTC |          |             |
| ABR0004     | 2          | CAATGGAATGCGAATTGAAC | 99       | (CA) 8       |
|             |            | GCAAATTTGATTCCTTGTT |          |             |
| ABR0008     | 2          | TAAGTGTAGCGACGGAAAG | 265       | (CA) 14      |
|             |            | CGCTGAGATGAAACAGGAGG |          |             |
| ABR0107     | 2          | CCGTTACTGACTCTGCTTT | 250       | (CA) 10      |
|             |            | TTTTATTGGCTCCCCCTAC |          |             |
| ABR0189     | 2          | TTAACATTAAAGGCGATCT | 109       | (CA) 9       |
|             |            | ATTTGAATCTCAAAAAACCT |          |             |
| ABR0153     | 2          | GAGACACCCCTACCTGCTGA | 263       | (CA) 8       |
|             |            | TACCCCTAATTGCCCCCAA |          |             |
| ABR0659     | 2          | AAGCAAAACGTTCCACCAA | 284       | (CA) 12      |
|             |            | GACTATCAAGATATTCACCAA |          |             |
| ABR0382     | 4          | CTGTGGTTGGAACCTTGAT | 226       | (CA) 9       |
|             |            | TGGAGAATCTGCGCTGATATT |         |             |
| ABR0622     | 4          | GAGCTCCACTCTACCCCATG | 231       | (CA) 15      |
|             |            | TCTTGCCAACTCAGTTCCTA |          |             |
| ADL0266     | 4          | AATGCGATTGAGGATGTG | 113–136   | (CA) 6       |
|             |            | GTGCGATTGAGGAGGCAG |          |             |
| ABR0392     | 5          | CGAAATACAGCCCTAGAAC | 138       | (CA) 9       |
|             |            | ATCCCTGTGAAAGGATCTA |          |             |
| ABR0399     | 5          | AGCCTAAAGCATTGGAACAC | 291       | (CA) 18      |
|             |            | GACAAGTCAAACACAGAAC |          |             |
| ABR0262     | 5          | ATAGCAGCAGGCTCAATGG | 279       | (CA) 9       |
|             |            | TTAATGAGTGAAGGGAAA |          |             |
| ABR0048     | 6          | ATTCTGCGGGACATCTGGAACAC | 91       | (CA) 11      |
|             |            | CTTGATCCTTTCCAGCTGTTGG |         |             |
| MCW0134     | 9          | GGAGCTCATTGTTGACAC | 284       | (CA) 24      |
|             |            | ACCAAAGACTGGAGGTCAAC |          |             |
| ABR0526     | 9          | TCAATTCAGATGCCTCCACA | 181       | (CA) 10      |
|             |            | GCAAAGGCTGCCATTACAT |          |             |
| ABR0495     | 10         | TTTGACTGAGGTGACATG | 249       | (CA) 15      |
be the major viral oncoprotein of GaHV-2. The MEQ interacts directly with p53 and inhibits p53-mediated transcriptional activity [25]. Both the MMR system and the p53 pathway are critical in the maintenance of genomic integrity [26,27].

A panel of five microsatellites has been validated and recommended as a reference panel for future research in the field at a National Cancer Institute Workshop meeting [8]. Tumors with instability at two or more of these markers were defined as being MSI-H (high-frequency MSI), whereas those with instability

| Marker name | Chromosome | Primer (5’-3’) | Size (bp) | Repeat array |
|-------------|------------|---------------|-----------|--------------|
| ACTCTTTGGCCTAATTTTCC | MCW0067 10 | GCACTACTGTGCTGAGTGTT | 178–184 | (CA) 11 |
| TAGGATAGTGCCACATTTGGCAC | ABR0325 10 | CATTTGTTTTTCTTTCTGAT | 156 | (CA) 18 |
| AAGTGGGACATCAACAGAG | ABR0389 11 | TTCCTCTATGCTAAGTCATCC | 231 | (CA) 12 |
| GGATTGACTCTTTGGCAGAC | ADL0308 11 | CCGCTGAACTGTGATGCTGTC | 164–165 | (CA) 13 |
| CTTGACATCTTTGGAATA | ABR0052 11 | CTGACAGAGCTCATAAGGTAAT | 209 | (CA) 9 |
| TCAGCTACTGTGCTGACAACA | ABR0059 12 | GAAACAAGCGAACGGCAACTAA | 192 | (CA) 9 |
| GGTTGGAACAGCGCGTGAAG | ABR0086 12 | GCTGAGGAGACGGCGTAA | 223 | (CA) 9 |
| CCCCCGAATGCTAAAGGATGCT | ABR0033 12 | AAGAGGAGGAGGGAAGGACAG | 220 | (CA) 9 |
| GCTTGGCGACGCATAACCCAG | LEI0099 12 | GATCTGCGAGACAGAAACAG | 131 | (CA) 12 |
| ATTTTCAGGCTGACCTGCG | ABR0634 12 | TACTGAATAAAAAGGAGAAC | 306 | (CA) 21 |
| CTGTTGAATGAAAGCAGTGA | ADL0147 13 | GCTGGCGACTAAAATCGCC | 211–220 | (CA) 8 |
| ACAAAGCCAAGCAGGCAAG | ABR0365 14 | ACAGTAACACTTTATGCAAT | 222 | (CA) 23 |
| AGCTAGGAAAGAGGAAATA | ABR0517 14 | GCAGGAGTGCCCTGGCAGGGT | 243 | (CA) 9 |
| GGCCACATCACAGCCCAGCT | ABR0387 17 | AATGGGTGTTGTGCTGAAAG | 288 | (CA) 14 |
| CTGGTTGCTGCCCACAAATGGG | ADL0199 17 | ACAAAGCCAAGGGAACACAT | 154–174 | (CA) 14 |
| GAGGAAGAGAGGACGCAGACC | ABR0650 18 | CGTAAAGGAAGCAGTAAATG | 318 | (CA) 11 |
| ATGGAAATGTGCTTGAGAG | ABR0133 19 | CCTGTTAATGTCTGCTTTG | 199 | (CA) 9 |
| GGAGGCCTTTCTGTGATTT | ABR0180 19 | ATGGAAATTTTACCCTGCTA | 145 | (CA) 11 |
| CGGCTATCTTATCCTGAGGATC | ABR0026 20 | AACATACAACTACAGGGGAAT | 193 | (CA) 10 |
| AGCGCTGGGTGCTGGGGTGA | ABR0123 20 | ACTCAACACGCTCAGTGCTA | 179 | (CA) 7 |
| CCATAACACCAAGGACCATCA | ABR0617 26 | CAAAGAATCTCATCAAGACAGGCA | 172 | (CA) 13 |

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at one, or showing no instability, were defined as MSI-L (low-frequency MSI) and MSS (microsatellite stable) tumors, respectively. If more than five markers are used to identify particular tumor phenotypes, then the criteria should be modified to assess the percentage of MSI rather than absolute number. The MSI-H group of tumors would be defined as having MSI in $\geq 30\%$, whereas the MSI-L group would exhibit MSI in $<30\%$. The diagnosis of MSI-H in cancers is becoming increasingly relevant; MSI-H is a useful screening marker for identifying some human tumors [28]. This was the first report of MSI in Marek's Disease lymphoma, and there is no reference to MSI-H/MSI-L markers for this tumor. Therefore, we recommend six markers (ABR0007, ABR0086, ABR0352, ABR0387, ABR0399, and MCW0145) as predictive biomarkers of Marek's Disease lymphoma as 40% (6/15) demonstrated MSI-H and 93% (14/15) of lymphomas showed MSI in the present study, according to the human criteria detailed above.

In conclusion, this study demonstrates that the phenomenon of MSI does occur in Marek's disease lymphoma induced by GaHV-2. Future studies will investigate the relationships among MMR, MSI and MD tumorigenesis to better understand the molecular nature of host genomic instability and virus infection.

Table 2. Number of MSI loci in different chickens.

| Chicken | Microsatellite markers | Chromosome | Number of MSI loci |
|---------|------------------------|------------|--------------------|
| 1       | ABR0352, ABR0522, ABR0622, ABR0086, ABR0365 | 1, 1, 4, 12, 14 | 5/30 |
| 2       | ABR0007, ABR0352       | 1, 5       | 2/30 |
| 3       | ABR0352               | 1          | 1/30 |
| 4       | ABR0107, ABR0399, ABR0133 | 2, 5, 19  | 3/30 |
| 5       | ABR0008, ABR0262, ABR0389, ABR0086 ABR0517 | 2, 5, 11, 12 | 5/30 |
| 6       | ABR0352, ABR0526       | 1, 9       | 2/30 |
| 7       | ABR0007, ABR0204, ABR0352, ABR0617 | 2, 10, 20, 26 | 5/30 |
| 8       | ABR0007, ABR0352, ABR0522, ABR0617 | 1, 1, 1, 1, 1, 11, 12,12,17 | 9/30 |
| 9       | ABR0007, ABR0352, ABR0522, ABR0526, ABR0617, ABR0634, ABR0387 | 1, 1, 1, 1, 1, 11, 12,12,17 | 9/30 |

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Table 3. Frequency of MSI for each microsatellite marker.

| Microsatellite markers | No. of Chickens | Frequency | Microsatellite markers | No. of Chickens | Frequency |
|------------------------|----------------|-----------|------------------------|----------------|-----------|
| ABR0007                | 2, 24, 29      | 3/15      | ABR0389                | 20, 25         | 2/15      |
| ABR0008                | 20             | 1/15      | ABR0399                | 2, 8, 25       | 3/15      |
| ABR0026                | 37             | 1/15      | ABR0517                | 20, 23         | 2/15      |
| ABR0033                | 24, 25         | 2/15      | ABR0522                | 1, 24          | 2/15      |
| ABR0052                | 24             | 1/15      | ABR0526                | 15             | 1/15      |
| ABR0086                | 1, 20, 21, 26  | 4/15      | ABR0617                | 29, 37         | 2/15      |
| ABR0107                | 37, 8          | 2/15      | ABR0622                | 1              | 1/15      |
| ABR0123                | 37             | 1/15      | ABR0634                | 24, 26         | 2/15      |
| ABR0133                | 8              | 1/15      | ABR0659                | 23, 26         | 2/15      |
| ABR0189                | 23             | 1/15      | ADL0147                | 23             | 1/15      |
| ABR0204                | 29, 38         | 2/15      | ADL0266                | 26             | 1/15      |
| ABR0262                | 20             | 1/15      | MCW0067                | 38             | 1/15      |
| ABR0352                | 1, 5, 23, 24, 29 | 5/15 | MCW0145                | 15, 24, 25, 26 | 4/15 |
| ABR0365                | 1              | 1/15      | LEI0099                | 25             | 1/15      |
| ABR0387                | 24, 37, 39     | 3/15      | LEI0146                | 24             | 1/15      |

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References

1. Pagano JS, Blaser M, Buendia MA, Damania B, Khalili K, et al. (2004) Infectious agents and cancer: criteria for a causal relation. Seminars in Cancer Biology 14: 453–471.
2. Damania B (2004) Oncogenic gamma-herpesviruses: Comparison of viral proteins involved in tumorigenesis. Nature Reviews Microbiology 2: 656–668.
3. Kaiser HE, Nasir A, Parcells MS, Burgess SC (2008) Immunological aspects of Marek's disease virus - Chicken host-pathogen interaction. Genomics of Disease: 115–126.
4. Lupiani B, Lee LF, Cui XP, Gimeno I, Anderson A, et al. (2004) Marek's disease virus: from miasma to model. Nature Reviews Microbiology 4: 283–294.
5. Cheng HH (2008) Integrating Genomics to understand the Marek's disease virus - Chicken host-pathogen interaction. Genomics of Disease: 115–126.
6. Lupiani B, Lee LF, Cui XP, Gimeno I, Anderson A, et al. (2004) Marek's disease virus-encoded Meq gene is involved in transformation of lymphocytes but is dispensable for replication. Proceedings of the National Academy of Sciences of the United States of America 101: 11815–11820.
7. Hag K, Brubin JF, Thanhtrng-ng Don N, Heidari M, Sharif S (2010) Transcriptome and proteome profiling of host responses to Marek's disease virus in chickens. Veterinary Immunology and Immunopathology 138: 292–302.
8. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, et al. (1998) A National Cancer Institute Workshop on Microsatellite Instability for Cancer Detection and Familial Predisposition: Development of International Criteria for the Determination of Microsatellite Instability in Colorectal Cancer. Cancer Research 58: 5248–5257.
9. Sanchez-Crepeda M, Monzo M, Rosell R, Pilaro A, Calvo R, et al. (1998) Detection of chromosome 3p alterations in serum DNA of non-small-cell lung cancer patients. Annals of oncology 9: 113–116.
10. Utting M, Werner W, Dahse R, Schubert J, Junker K (2002) Microsatellite analysis of free tumor DNA in urine, serum, and plasma of patients: a minimally invasive method for the detection of bladder cancer. Clinical cancer research 8: 35–40.
11. Yoon B-S, Kim Y-T, Kim J-H, Kim S-W, Nam E-J, et al. (2008) Clinical significance of microsatellite instability in sporadic epithelial ovarian tumors. Yonsei Medical Journal 49: 272–278.
12. Yee CJ, Roodi N, Verrier CS, Parm FF (1994) Microsatellite instability and loss of heterozygosity in breast cancer. Cancer research 54: 1641–1644.
13. Chang MS, Lee JH, Kim HS, Lee HS, et al. (2000) Microsatellite instability and Epstein-Barr virus infection in gastric remnant cancers. Pathology International 50: 486–492.
14. Chang MS, Lee HS, Kim HS, Kim SH, Choi SI, et al. (2003) Epstein-Barr virus and microsatellite instability in gastric carcinomas in Hong Kong Chinese. Britsh Journal of Cancer 79: 582–586.
15. Wu MS, Shun CT, Wu CC, Hsu TY, Lin MT, et al. (2000) Epstein-Barr virus - Associated gastric carcinomas: Relation to H-pylori infection and genetic alterations. Gastroenterology 118: 1031–1038.
16. KoHAVONG P, Grant SG, Saha A, Baira AK, Bamezai R (2005) Microsatellite Instability. Molecular Toxicology Protocols: Humana Press. 293–302.
17. Leung SY, Yuan ST, Chung JP, Chu KM, Wong MP, et al. (1999) Microsatellite instability, Epstein-Barr virus, mutation of type II transforming growth factor beta receptor and BAX in gastric carcinomas in Hong Kong Chinese. British Journal of Cancer 79: 582–586.
18. Shang Y, Qiao LP, Han T, Xia J, Yang D, et al. (2009) Microsatellite instability on Chromosome 6 of chicken embryo fibroblast infected with Marek's disease virus. Journal of Nanjing Agricultural University 32: 115–118.
19. Sanguinetti CJ, Dias Neto E, Simpon AJ (1994) Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. BioTechniques 17: 914–921.
20. Araimazoglou, H, Gilbert F, Barber HRK (1990) Microsatellite instability in human solid tumors. Cancer 82: 1800–1820.
21. Aithodis DK, Reddy SM, Suchodolof PK, Lee LF, Kung HJ, et al. (2009) In vitro characterization of the Meq proteins of Marek's disease virus vaccine strain CVI988. Virus Research 142: 57–67.
22. Miturski R, Bogusiewicz M, Ciotta C, Bignami M, Gogacz M, et al. (2002) Mismatch repair genes and microsatellite instability as molecular markers for gynecological cancer detection. Experimental Biology and Medicine 227: 579–587.
23. Fishel R, Kolodner RD (1995) Identification of Mismatch Repair Genes and their Role in the Development of Cancer. Current Opinion in Genetics & Development 5: 382–395.

Author Contributions

Conceived and designed the experiments: DWY, JRX, DJY. Performed the experiments: DWY, JRX, STL. Analyzed the data: DWY, JRX, ZLZ. Contributed reagents/materials/analysis tools: DWY, JRX, ZLZ. Wrote the paper: DWY, ZLZ, DJY.
24. Li ZJ, Qin AJ, Qian K, Chen XH, Jin WJ, et al. (2010) Proteomic analysis of the host response in the bursa of Fabricius of chickens infected with Marek’s disease virus. Virus Research 153: 250–257.
25. Deng XF, Li XD, Shen Y, Qiu YF, Shi ZX, et al. (2010) The Meq oncoprotein of Marek’s disease virus interacts with p53 and inhibits its transcriptional and apoptotic activities. Virology Journal 7: 348.
26. Hoffmann JS, Gazeaux C. (1998) DNA synthesis, mismatch repair and cancer. International Journal of Oncology 12: 377–382.
27. Honma M, Momose M, Tanabe H, Sakamoto H, Yu YJ, et al. (2000) Requirement of wild-type p53 protein for maintenance of chromosomal integrity. Molecular Carcinogenesis 28: 203–214.
28. Imai K, Yamamoto H (2008) Carcinogenesis and microsatellite instability: the interrelationship between genetics and epigenetics. Carcinogenesis 29: 673–680.