Gynecologic Oncology 138 (2015) 358–362

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Characterization of Toll-like receptor transcriptome in squamous cell carcinoma of cervix: A case–control study

Ritu Aggarwal [a,*], Sunayana Misra [a], Charu Guleria [a], Vanita Suri [b], Navdeep Mangat [a], Madhulika Sharma [a], Raje Nijhawan [c], Ranjana Minz [a]

[a] Department of Immunopathology, Post Graduate Institute of Medical Education and Research, Chandigarh, India
[b] Department of Obstetrics and Gynecology, Post Graduate Institute of Medical Education and Research, Chandigarh, India
[c] Department of Cytology and Gynecologic Pathology, Post Graduate Institute of Medical Education and Research, Chandigarh, India

HIGHLIGHTS

- A significant down-regulation in the gene expression of TLRs 3, 4 and 5 was observed in cervical squamous cell cancer.
- A significant up-regulation in the gene expression of TLR 1 was observed in cervical squamous cell cancer.
- Study results evoke the proposition of investigating TLRs 3, 4 and 5 agonists for therapeutic exploration.

ARTICLE INFO

Article history:
Received 29 April 2015
Received in revised form 23 May 2015
Accepted 25 May 2015
Available online 27 May 2015

Keywords:
Toll like receptors
Cervical cancer
Human papillomavirus
TLR
India
Polymerase chain reaction

ABSTRACT

Objective. Human papillomavirus (HPV) is a proven etiological agent for cervical cancer However, not all HPV infections result in cervical cancer. The mechanisms of host immune system to prevent/control HPV infection remain poorly understood. Toll-like receptors (TLRs) are a system of innate immune defense. HPV has been demonstrated to modulate TLR expression and interfere in TLR signaling pathways, leading to persistent viral infection and carcinogenesis. The aim was to study the relative gene expression of TLRs in cervical squamous cell carcinoma (SCC).

Methods. Gene expression profile of TLRs 1 to 9 was examined in 30 cervical SCCs and an equal number of normal cervical tissue samples using a PCR array platform. Gene expression studies for TLRs 3 and 7 were validated by western blotting.

Results. HPV was detected in all cases and in none of the controls ($p < 0.0001$). HPV16 was the preponderant (83.3%) subtype. A significant downregulation in the relative gene expression of TLR3 ($p < 0.0001$), TLR4 ($p < 0.0005$) and TLR5 ($p < 0.0001$) was observed in cases. A significant upregulation for TLR1 was observed ($p = 0.006$). Although TLRs 2, 7, 8 and 9 were upregulated and TLR6 was downregulated, it was not significant. The western blot performed with antibodies against TLRs 3 and 7 confirmed the findings of the gene expression studies.

Conclusions. A significant downregulation in the gene expression of TLRs 3, 4 and 5 and upregulation of TLR1 was observed in cervical SCC as compared to controls. Study results evoke the proposition for investigating TLRs 3, 4 and 5 agonists for therapeutic exploration.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Cervical cancer is the second most common cancer in females worldwide and the most common cancer in Indian women [1]. Cervical cancer progresses gradually in a multistep process which involves the transformation of normal cervical epithelium to pre-invasive cervical intraepithelial neoplasia (CIN), to invasive cancer [2]. Human papillomavirus (HPV) is well established as an etiological agent for cervical cancer. However, infection with HPV by itself is thought to be insufficient for the malignant transformation of HPV infected cell [3]. Persistent infection with HPV along with chronic inflammation can induce cancer formation via cytokines and chemokines [4]. Toll-like receptors (TLRs) recognize conserved molecules associated with microbial pathogens, known as pathogen-associated molecular pattern (PAMP) which leads to the production of different types of cytokines and activation of adaptive responses against several pathogens [5]. In recent years, it has been found that TLRs are not only expressed in immune cells, but in tumor cells as well [6]. TLR expressed tumor tissues and cells hinder the infiltration of immune cells. They can also change the type of inflammation, therefore facilitate tumor occurrence and development [7].

* Corresponding author.
E-mail address: ritu_immunopath@yahoo.co.in (R. Aggarwal).

http://dx.doi.org/10.1016/j.ygyno.2015.05.029
0090-8258/© 2015 Elsevier Inc. All rights reserved.

TLRs may have a critical role in tumorigenesis; they can create an environment conductive for carcinogenesis through inflammatory response, angiogenesis and cell death [10]. Although limited data is available, it is clear that interplay between HPV and the host TLR signaling pathway exists [3]. HPV may invade innate immunity by avoiding TLR recognition [3]. As a result, TLRs establish a suitable microenvironment for tumor cell growth that allows tumor cells to evade immune cells, infiltrate, metastasize and undergo malignant progression [8].

The aim of the study was to explore the relative gene expression of TLRs in cervical squamous cell carcinoma (SCC) as compared to normal cervical tissue. Various TLR agonists are currently under clinical trial to investigate anti-tumor immunity [9]. Understanding the expression of TLRs in cervical cancer will likely aid in exploring therapeutic avenues.

2. Materials and methods

The study was a single-center, prospective, case–control study. Women with cervical growth, confirmed on histopathology as SCC of cervix were enrolled. Enrollment was performed randomly, i.e., by a non-consecutive, convenience sample. Treatment naïve patients were recruited. Women undergoing hysterectomy for non-cervical and non-malignant indications were included as controls. Age at marriage was enquired; age at first sexual intercourse was not asked, as the question is not considered socially/culturally appropriate. Socioeconomic status was evaluated by modified Kuppuswamy's socioeconomic scale [10]. The study was approved by the institute's ethics committee. Informed consent was obtained from the study participants.

2.1. Sample collection

In the Gynecology outpatient, along with the diagnostic biopsy, a small piece of the growth was collected. It was immediately transported to the laboratory and preserved at minus 80 °C, till further processing. Cases confirmed on histopathology as SCC of cervix were processed for the study. Controls were confirmed to have normal cervix on histopathology.

2.2. DNA extraction

DNA was extracted from the tissue biopsy with a commercially available extraction kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions. The quality of DNA was confirmed by amplification with polymerase chain reaction (PCR), using primers for beta-actin, a housekeeping gene.

2.3. HPV genotyping

Genotyping of HPV was carried out with HPV genotyping array kit (Hybribio, Hong Kong, China), a PCR based assay. It is capable of detecting 21 HPV genotypes simultaneously (high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68), 2 probable high-risk types (53 and 66) and 6 low-risk types (6, 11, 42, 43, 44, and 81). Amplification was performed with L1 consensus primers for HPV genotypes, followed by flow-through hybridization with immobilized genotype-specific probes. Each test included two controls: a) biotin dot: positive control for the detection reaction of the enzyme conjugate, and b) internal control dot: for monitoring the PCR amplification. In addition, a positive (HPV18 plasmid DNA) and a negative control were included for quality check. The positive result was read visually as a purple precipitate at the probe dot.

2.4. RNA isolation and cDNA synthesis

Total RNA was isolated from the sample of cervical biopsy with a commercially available kit (RiboPure™, Ambion, Life Technologies, USA) according to the manufacturer's instructions. The yield and purity of RNA was assessed by spectrophotometer by measuring absorbance at 260 nm and 280 nm. Integrity of RNA was verified by denaturing agarose gel electrophoresis, followed by staining with ethidium bromide. RNA bands on the gel were visualized under UV light; the presence of 18S as well as 28S rRNA bands confirmed good quality. A total RNA measuring 500 ng was used to generate first strand cDNA as initial step of a two-step reverse transcription (RT) PCR. Contamination with genomic DNA was eliminated by the pretreatment of cellular RNA with DNase1 (Invitrogen, San Diego, CA). Synthesis of cDNA was performed using RT^2 first strand kit (Qiagen, Germany) according to the manufacturer's instructions. cDNA of each sample was amplified using primers of beta-actin to check the efficiency of reverse transcription.

2.5. Relative gene expression assay by RT-PCR

Quantitative PCR was performed using customized RT^2 profiler PCR array (SABiosciences, Maryland, USA). The array platform consisted of: a) primer assays for TLR genes — 1 to 9, b) a housekeeping gene to normalize the data, c) a well containing a genomic DNA control to detect contamination with non-transcribed genomic DNA, d) a well containing reverse transcription control to assess the efficiency of reverse transcription reaction, and, e) a well containing positive PCR control to examine the efficiency of PCR. The analysis of data was performed using the ΔΔCt method. Relative quantification of genes was carried out on LightCycler® 480 RT-PCR system (Roche Diagnostics, Germany). The cycle threshold values obtained were exported for analysis. The examination of the raw data was performed using the SABiosciences data analysis software (SABiosciences, Maryland, USA).

2.6. Western blot

The tissue from cases as well as controls was lysed with lysis buffer, containing RIPA buffer and protease inhibitor. The protein concentration was determined using bicinchoninic acid assay reagent (Pierce Biotechnology, Rockford, lL, USA). Ten micrograms of total protein sample was denatured at 100 °C for 5 min. Proteins were separated on 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membrane. The membrane was blocked with 5% skimmed milk in TBST (mixture of tris-buffered saline and tween 20) for 1 h at room temperature. The membranes were incubated with anti-mouse TLR3, TLR7 (Santa Cruz Biotechnology, Inc., Dallas, Texas, USA) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Ambion, Austin, TX, USA) antibodies at 1:1000 dilution in TBST overnight at room temperature. Subsequently, membranes were incubated with horseradish peroxidase conjugated secondary antibody (Merck, Germany) at 1:2000 dilution for 1 h at room temperature. Visualization was done by enhanced chemiluminescent substrate (Bio-Rad laboratories, CA, USA). GAPDH served as internal control.

2.7. Statistical analysis

Data analysis was performed with SPSS Statistical Package, version 17, for PC. Baseline variables were analyzed by descriptive statistics. Comparison of proportions was done with chi-square test. The one-sample t-test was utilized to compare TLR gene expression between cases and controls. The student's t-test was used to study the demographic variables. A p-value < 0.05 was considered statistically significant.

3. Results

Thirty women with histologically confirmed SCC of cervix formed the case-cohort. Equal number of women with a normal cervix was enrolled as controls. The list of diagnosis in women in the control group included, fibroid uterus (83.3%), adenomyosis (10%), and

Download English Version:

https://daneshyari.com/en/article/3943160

Download Persian Version:

https://daneshyari.com/article/3943160

Daneshyari.com