Interaction between Interferon Regulatory Factor-1 and Human Papillomavirus E7 Oncogene in Cervical Cancer: An Ontology Study

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SUMMARY

Objective: Cervical cancer is an important female malignancy. The discovery of human papillomavirus (HPV) as an etiologic agent of cervical cancer has prompted increased interest in the biology and oncogenicity of this virus. The E7 protein is found predominantly in the nucleus and, to a lesser extent, in the cytoplasm in cervical cancer cell lines. HPV E7 has been shown to be functionally associated with the tumor suppressor interferon regulatory factor (IRF)-1 in cervical carcinogenesis.

Materials and Methods: In this study, new gene ontology technology was used to predict changes in the molecular function and biologic processes caused by the interaction between IRF-1 and HPV E7.

Results: The molecular function and biologic processes of IRF-1 and the combined IRF-1 and HPV E7 (IRF-1-E7) were derived using the GoFigure server. The combined IRF-1-E7 demonstrated more functions and biologic processes compared with IRF-1 alone.

Conclusion: IRF-1-E7 was shown to be responsible for the positive regulation of many interleukins and to be involved in the differentiation of T-helper cells. [*Taiwan J Obstet Gynecol* 2009;48(2):138–141]

Key Words: cervical cancer, E7 oncogene, human papillomavirus, interferon regulatory factor-1 suppressor protein

Introduction

Cervical cancer is an important female malignancy and has been reported to be the third most common cancer affecting women [1]. Although cervical cancer is not a sexually transmitted disease, it is related to the presence of the human papillomavirus (HPV), which is sexually transmitted [1]. About 500,000 cases of cervical cancer are registered every year worldwide, of which an estimated 80% occur in developing countries [2]. The World Health Organization has estimated that 7 million new cancer cases occur every year, at least half of which are in low-income, developing countries [2]. The discovery of HPV as an etiologic agent for cervical cancer has generated increased interest in the biology and oncogenicity of this virus [3]. Birley [3] proposed that HPV infection could be an important factor contributing to the high prevalence of cervical carcinoma in developing countries.

The HPV E7 protein can be detected predominantly in the nucleus and, to a lesser extent, in the cytoplasm in cells of the cervical cancer cell line, Ca Ski, *in vitro*, and in invasive cervical carcinoma *in situ*, suggesting that nuclear E7 plays a major role in cervical carcinogenesis in humans [4]. HPV E7 has been reported to be functionally associated with the tumor suppressor interferon regulatory factor (IRF)-1 in cervical carcinogenesis [5]. Binding assays indicate a physical interaction between IRF-1 and HPV E7 *in vivo* and *in vitro* [5]. Um et al [6] found that E7 transgene expression

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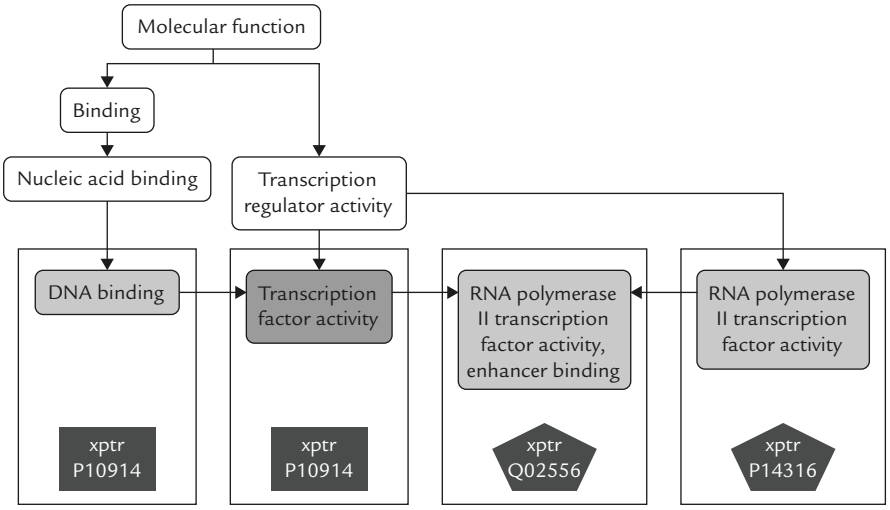

Figure 1. Expected molecular function of interferon regulatory factor-1.

inactivated the transactivation function of IRF-1 *in vivo,* which could be an important aspect of its role in cancer development. It is difficult to study the interaction between these two proteins during carcinogenesis, but new developments in bioinformatics can be applied to nanoscale genomics and proteomics research. In this study, a new gene ontology technology was used to predict changes in the molecular function and biologic processes caused by the interaction between IRF-1 and HPV E7.

Materials and Methods

Determination of amino acid sequences for IRF-1 and HPV E7

The database PubMed was used for data mining to access the amino acid sequences of IRF-1 and HPV E7.

Prediction of molecular function and biologic processes

A novel gene ontology prediction tool, GoFigure, was used to predict the molecular function and biologic process of IRF-1 and of the combination of IRF-1 and HPV E7 (IRF-1-E7) [7]. GoFigure is a computational algorithm tool which has been recently developed for gene ontology studies [7]. This tool accepts input DNA or protein sequences and uses BLAST to identify homologous sequences in gene ontology-annotated databases. It is also possible to use a BLAST search to identify homologs in public databases that have been annotated with gene ontology terms, including SWISS-PROT, FlyBase (*Drosophila*), the *Saccharomyces* Genome Database (SGD), Mouse Genome Informatics (MGI), and WormBase (nematode) [7]. Results for molecular function, as well as for biologic processes of the studied protein, can be predicted [7]. The predicted molecular functions and biologic processes of IRF-1 and IRF-1-E7 were presented and compared in this study.

Results

The molecular function and biologic processes of IRF-1 and IRF-1-E7 are presented in Figures 1 and 2. The molecular functions and biologic processes of IRF-1 and the combination of IRF-1-E7 are compared in the Table.

Discussion

A strong association between cervical cancer and high-risk HPV-16 and -18 infections underlines the importance of the virus in the pathogenesis of many squamous cell carcinomas [8]. High-risk HPV infection can lead to carcinogenesis and tumor progression, predominantly through the actions of the viral oncogene, E7 [8]. Constitutive activation of the telomerase is a key step in the development of human cancers [9]. Interferon (IFN)-γ signaling induces growth arrest in many tumors through multiple regulatory mechanisms. Lee et al [9] noted that IFN-γ signaling repressed telomerase activity and human telomerase reverse transcriptase transcription, and suggested that this signaling was mediated by IRF-1. They also observed less telomerase repression in HPV E7-negative, p53-mutant HT-3 cells, compared with HPV-18 E6- and E7-positive HeLa cells [9]. Park et al [5] suggested that HPV E7 interfered with the transactivation function of IRF-1 by recruiting histone deacetylase

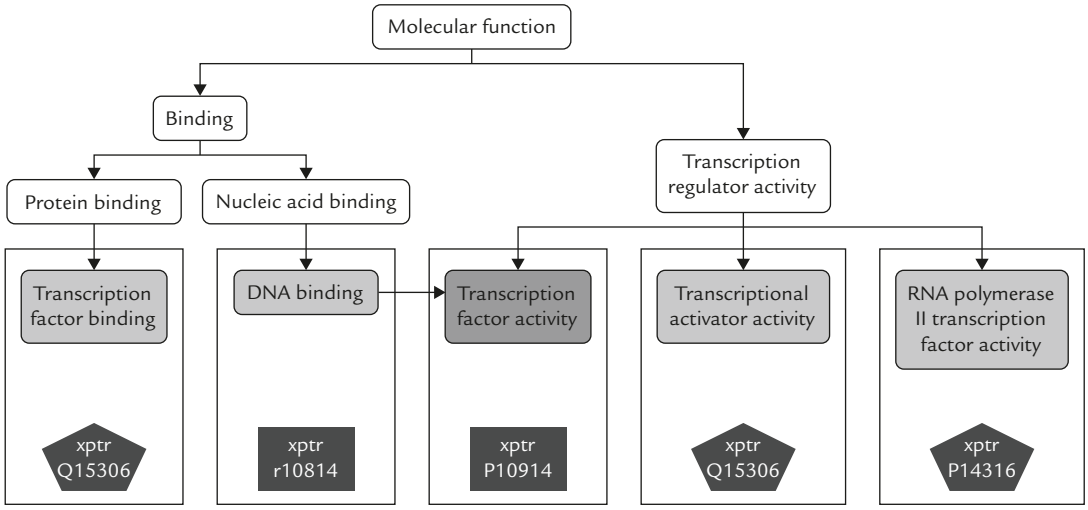

Figure 2. Expected molecular function of the combined interferon regulatory factor-1 and human papillomavirus E7.

Table. Comparison of the molecular functions and biologic processes of interferon regulatory factor (IRF)-1 and the combined IRF-1 and HPV E7 (IRF-1-E7)

Proteins	Molecular function	Biologic process
IRF-1	DNA binding, transcription factor activity RNA polymerase II transcriptional factor activity RNA polymerase II transcriptional factor activity (enhancer-binding)	Positive regulation of transcription (DNA-dependent) Negative regulation of transcription from Pol II promoter Positive regulation of interleukin-12 biosynthesis
IRF-1-E7	DNA binding Transcription factor activity RNA polymerase II transcriptional factor activity Transcription factor binding Transcriptional activator activity	Negative regulation of transcription from Pol II promoter Positive regulation of interleukin-13 biosynthesis Positive regulation of interleukin-4 biosynthesis Positive regulation of interleukin-12 biosynthesis Positive regulation of interleukin-2 biosynthesis Positive regulation of interleukin-10 biosynthesis Regulation of T-helper cell differentiation

to the promoter, and they proposed that the interaction between IRF-1 and HPV E7 was important for carcinogenesis.

Based on recent advances in genomic technology, current microarray techniques can be used to analyze the gene expression patterns of tens of thousands of genes [7]. Gene ontology has been developed for this specific purpose, and many gene ontology tools have been constructed and launched. In this study, a gene ontology tool was used to predict the functions of IRF-1 and IRF-1-E7. Compared with IRF-1, IRF-1-E7 uncovered many different functions and biologic processes, including the positive regulation of many interleukins. Kyo et al [10] proposed that inflammatory cytokines contribute to the host's defense against HPV infection. However, Iglesias et al [11] found that HPV E7 RNA expression and apoptosis increased in parallel in proliferating keratinocytes in severe dysplasias and carcinomas, suggesting that interleukin release was associated with progression to high-grade disease. They also noted that high-level expression of the HPV E7 protein sensitized keratinocytes to apoptosis, resulting in release of interleukins, multifunctional cytokines that promote inflammation, tissue remodeling and epithelial hyperplasia [11].

Interestingly, IRF-1-E7 also appeared to be involved in the differentiation of T-helper cells in this study. According to a recent study, disease recurrence was accompanied by an increase in the total number of specific short-term T-cell lines at follow-up, whereas absence of disease was accompanied by a decrease in specific short-term T-cell lines [12]. In addition, HPV-specific memory cytotoxic T-cell (non-T-helper cell) precursors were detected, predominantly in women with high-grade lesions or invasive cervical carcinomas, and not in women who were completely clear of the virus.

Further studies are needed to clarify the role of IRF-1-E7 in the process of differentiation of T-helper cells.

Further experimental studies are needed to confirm the role of RIF-1-E7. The findings of this study, however, not only support the results of previous studies of HPV and cervical carcinoma, but also provide new insights into the interaction between RIF-1 and HPV E7. There were some limitations to this work; because of the nature of ontology studies in bioinformatics, schematic figures and explanations were provided to show the possible interaction between the two molecules. However, further laboratory studies are needed to provide detailed evidence for the actual nature of this specific protein–protein interaction. An *in vitro* protein–protein pull-down assay should be performed to determine the biologic reaction implied by the results of this ontology study.

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