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Review and Meta-analysis

Molecular and Potential Biomarkers in Diagnosis of Cervical Carcinoma: A Review

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Cervical cancer, a potentially preventable disease remains the second most common malignancy in women worldwide. Persistent infection with oncogenic human papillomavirus (HPV) is the main risk factor for cervical cancer and its precursor lesions. It starts in the cell lining of the cervix which includes abnormal cell division and functions. Cytological screening programs using the Pap test have led to a substantial reduction in the incidence of cervical carcinoma. The identification of new biomarkers that allows monitoring of molecular events associated to tumour progression in histological or cytological specimens may improve the detection of lesions with higher risk of progression. In the present article we review on molecular and potential biomarkers that interfere in the pathophysiology of disease and their possible role in screening and diagnosis of cervical cancer.

Introduction

Cervical cancer is one of the principal causes of morbidity and mortality among women worldwide. It ranks as the second most leading common malignancy among women [1]. Cervical cancer is the growth of abnormal cells in the cell lining of the cervix-the lower part of the uterus(womb) [2]. Human papillomavirus (HPV) infection with high risky type is the single most important etiological agent in cervical cancer, contributing to neoplastic progression through the actions of viral oncoproteins, mainly E6 and E7 [5]. The occurrence of cervical cancer is reported in women aged between 15 and 45 years. It is estimated to cause over 4,70,000 new cases and 2,33,000 deaths for each year [3]. A high incidence of this carcinoma is seen in low-income countries especially in Africa, Latin America and parts of Asia due to lack of effective screening programs and a high prevalence of HPV [4]. It is estimated that for every 1 million women infected, a hundred thousand (about 10%) will develop precancerous changes in their cervical tissue. Of these about 8% of them will develop early carcinoma limited to cervical epithelium and a few of them will develop invasive cancer unless the precancerous lesions are detected and treated with such cases having been found to carry the oncogenic HPVs that causes cervical cancer [5].

Cervical Cancer Screening

- Papanicolaou test (PAP smear test)

- Cervicography

- Speculoscopy

- Visual inspection with acetic acid (VIA)

- Liquid-based cytology

- HPV DNA test

- Colposcopy

- Cervical cytology

- Biopsy

- Computed tomography scan

- Bimanual pelvic examination under anaesthesia

- X-ray

For more than half a century, the PAP smear has served as the cornerstone of cervical screening, resulting in a remarkable decline in cervical cancer- related fatalities. Nevertheless, this screening technique has certain limitations, including its low sensitivity and a high rate of false negative results. As a result, several visual tests have been investigated as potential primary screening methods or as supplementary tests to cytology- based screening. These tests encompass cervicography, visual inspection with acetic acid (VIA) and speculoscopy. However, at present cervicography plays a limited role as either a primary screening tool or an adjunct to the PAP smear [6]. Presently, new technologies such as liquid-based cytology, HPV DNA tests have been introduced. This test is used to detect HPVs infection, which is considered as the primary cause of all cervical cancers. There are at least 30 different types of HPV strains that targets the genital area, and are transmitted through sexual, skin to skin contact. Although the pap smear can pick up the cellular changes by high-risk HPV, it is not as sensitive as HPV test, which specifically detects the viral DNA. The HPV test is not yet routinely used by majority of doctors because it is more expensive than a regular pap test. Therefore, it should be must to improve the cost effectiveness of screening and reduce the psychologic burden of benign positive test

results [7].

Materials and Methods to Determine the Cervical Carcinoma

Gene expression datasets of cervical cancer

In order to examine the gene expression patterns in cervical cancer, five distinct transcriptome datasets (GSE7803, GSE9750, GSE39001, GSE2903 andMGSE63514) containing information from cervical epithelium samples were acquired from the gene expression Omnibus (GEO) database. To prevent unwanted variations due to variations in microarrays, exclusively Affymetrix microarrays were utilized [8] (Table 1).

GEO ID	#of Tumour samples	HPV type	#of control samples
GSE7803	21	HPV16:10, HPV18:4, HPV18/45:1, HPV33/52/58:4, HPV58:1, HPV59:1	10
GSE9750	33	HPV16:19, HPV18:3, HPV45:4, HPV16/18:1, HPV18/45:1, HPV16/31/45:2, HPV16/18/31/45:1, HPV:2	24
GSE39001	19	HPV16: 19	5
GSE52903	55	HPV16: 55	17

GSE63514	28	HPV16:19, HPV18:1, Unspecified:8	24

Table 1. Transcriptome Datasets Employed in Present Study.

Identification of differentially expressed genes

A previously conducted statistical analysis procedure was adopted in present study to determine the DEGs. The raw data in files of each dataset were normalised by calculating the Robust Multi-Array average (RMA) expression measure as implemented. DEGs were identified from expression values by using linear models for Microarray Data (LIMMA) package. The Benjamini-Hochberg method was used to control the false discovery rate. To determine the statistical significance, adjusted $p < 0.01$ was used. Further analyses were performed with mutual DEGs among all 5 datasets called 'the core genes of cervical cancer' [9].

Gene set over representation analyses

These analyses were performed using the DAVID bioinformatics tool to identify functional annotations (i.e., biological processes, molecular functions, signalling and metabolic pathways, diseases) significantly associated with the core genes of cervical cancer [9].

Reconstruction of protein-protein interaction networks and topological analysis

The physical interactions of proteins encoded by core genes of cancer were analysed by reconstruction of PPI networks. For this, high confidence human protein interactome was employed. PPI networks were reconstructed around down and up regulated genes separately and represented as undirected graphs in cytoscape [9].

Identification of reporter metabolites associated with cervical cancer

To identify reporter metabolites around which significant transcriptional changes occur, the statistically significant changes in gene expression profiles were mapped onto the Human Metabolic Reaction (HMR 2.0) model through the reporter metabolites algorithm implemented in the Biomet Toolbox. The over representation of reporter metabolites in metabolic pathways was determined using pathway annotations presented by Metabolites Biological Role (MBRole) database [9].

Identification of reporter receptors, transcription factors and miRNAs

The reporter features algorithm was adopted and implemented in MATLAB (R2010) to identify reporter receptors, TFs, and miRNAs. The original algorithm was integrated differential transcription data with a metabolic model to identify metabolites. The metabolites with highest scores were identified as reporters.

In this study, we adapted algorithm to identify receptors by using a receptor-protein interaction network. for this purpose, the proteins that have receptor activity (GO:0004872) were screened in DAVID, PANTHER, Genecodis databases [9].

Cross-validation of the reporter biomolecules

The prognostic power of reporter biomolecules (i.e., 10 hubs, 18 receptors, 3 TFs, and 16 miRNAs) was analysed at transcriptome level by using independent gene expression (RNA-Seq or miRNA-

Seq) datasets obtained from The Cancer Genome Atlas (TCGA). The RNA-seq dataset consists of 191 samples with clinical information.

The subjects were partitioned into low-high risks groups according to their prognostic index, survival multivariate analyses and risk assessments were performed by SurvExpress [9].

Molecular Biomarkers in Cervical Cancer (Figure 1)

Figure 1. The Above Figure Displays the Number of Molecular and Potential Biomarkers Employed for Diagnosis and Helps in Treating Cervical Cancer in Women.

1. HPV E6

- The E6 oncoproteins of high-risk HPV interfere with the function of the cellular tumour suppressor protein p53 through the induction of increased proteasome-dependent p53 degradation.

- High risk HPV E6 proteins target the cellular E3 ubiquitin ligase E6-AP to p53, resulting in transfer of ubiquitin peptides from E6-AP to p53 for degradation by the 26S proteasome.

- Low risk and cutaneous epithelia-infecting HPV E6 proteins are unable to target the cellular p53 protein for degradation through the proteasome pathway. Although E6-induced loss of p53 is an important element of E6 that may also play an important role [10].

- Cellular binding partners for HPV E6 are: GAP, E6TP1, E6BP(ERC55), hDlg, MUPP1, BAK, ADA3, BAX, PKN, MAGI-1/2/3, CBP/p300, hMCM7, Gps2, FADD, IRF3, TNFR1, hScrib E6AP, p53, MGMT, XRCC1, myc, paxillin, Fibulin-1, Zyxin [10].

2. HPV E7

- HPV E7 Proteins interact with so called ‘pRb- associated pocket proteins’ including the retinoblastoma protein pRb, which are negative between cell cycle regulators involved in G1/S and G2/M transitions.

- The interaction between high-risk E7 pRb results in enhanced phosphorylation and degradation. pRb destruction leads to release of E2F family of transcription factors and subsequent activation of genes promoting cell proliferation.

- Cellular binding partners of HPV E7 are: pRb, Cyclin A, E, p21cip1, p27kip1, AP-1, p48, IRF-1, IRF-9, Mpp2, TBP, TAF110, Mi2, S4 subunit, hTid-1, IGFBP-3, Histone H1 kinase, Smad proteins 1-4, M2 pyruvate kinase, pRb- pocket proteins, A-glucosidase [10].

3. Mini chromosome maintenance [MCM]

- DNA replication occurs only once in a single normal cell cycle, due to a mechanism known as ‘licensing’ of DNA replication. This process requires assembly of a protein complex which includes the MCM proteins and the cell division cycle protein 6 (CDC6) [11].

- Disassembly of this complex prevents repetitive replication during the same cell cycle. Changes in expression pattern of DNA ‘licensing’ proteins are frequently observed in dysplastic cells.

- In normal cervical epithelium, MCM protein staining is limited to basal proliferating layer and is absent in differentiated and quiescent cells.

- The expression is dramatically increased, suggesting its potential as a biomarker of cervical dysplasia. It is a highly informative marker of cervical cancer [12].

4. Cell division cycle protein 6 [CDC6]

- Both MCM5 and CDC6 plays essential roles in the regulation of eukaryotic DNA replication.

- CDC6 was first identified in 1998 as a marker of cervical dysplastic cells in cervical biopsies and in smears using polyclonal antibodies [14].

- In normal cervical epithelium, CDC6 staining is absent or limited to the basal proliferative layer. Several studies have illustrated a linear increase in CDC6 expression in areas exhibiting histological HPV changes.

- Interestingly, the expression pattern of CDC6 closely mirrors that of high-risk HPV E6 oncoprotein, which is mainly expressed in higher grade lesions and invasive carcinomas [12].

5. p16INK4A

- It is a tumour suppressor gene and a key regulator of the cell cycle. The expression pattern of p16INK4A in dysplastic squamous and glandular cervical cells in tissue sections and in cervical smears has been extensively investigated [13].

- It identifies the invasion lesions with a sensitivity rate of 99.9% and a specificity rate of 100% in cervical biopsy sections.

- It is a specific and surrogate marker of high-risk HPV virus, suggesting a valuable adjunctive test in cervical screening [14].

6. Squamous cell carcinoma antigen [SCC]

- SCC belongs to the family of serine and cysteine protease inhibitors.

- This antigen is present in normal cervical epithelium in proportion to dysplastic lesion and cervical squamous cell carcinoma [15].

- Though SCC is not sufficient for use in screening, pre-treatment serum scc values works as an independent prognostic factor [16].

- Approximately 60% of patients with cervical cancer are detected with elevated levels of serum scc at initial diagnosis. Besides, serum SCC levels correlate significantly with tumour stage [17].

- Patients with plateau SCC level revealed higher incidence of treatment failure after radiotherapy, indicating scc levels provide useful information for further management. Clinically SCC is suitable for monitoring early detection of recurrent disease after primary treatment [18, 19].

New Biomarker Discovery and Development

- Although few new markers have reached the clinic in recent years, several reported cancer biomarkers have been found to have low sensitivity [20].

- In future the clinical cancer management belongs to prognostic and predictive markers of cancer, they are important as they will be used to make clinical decisions that may save lives [21].

- Biomarkers that correctly predict the outcome in a specific disease and allow physicians and patients to make informed decisions for treatment need to be developed [22].

- It should be concerned as whether the tools available are well suited to provide the technological support to meet demands of new biomarker development [19].

- The discovery of biomarkers has been a slow approach to identify proteins that are dysregulated as a sequence of disease and shed into body fluids, such as serum, saliva, urine [23].

- The recent advancements in genomic technologies improved new mass spectrometric technologies with advanced bioinformatic tools. Those shows great promise of meeting demand for a variety of new biomarkers discovery [24, 25].

- The combined use of genomics, proteomics and bioinformatics tools may hold promise for early detection of disease by proteomic patterns [24].

- Diagnosis based on proteomic signatures as a compliment to histopathology [26].

- Individualized selection of therapeutic combinations that targets the entire disease specific protein network, rational modulation of therapy based on changes in diseased protein associated with drug resistance and understanding of carcinogenesis [25].

Challenges in biomarker development

- A number of challenges can be occurred in biomarker discovery to development.

- Oncologists and scientists are aware that validation and implementation in clinic biomarkers is long and complicated [27, 28].

The main challenges included are as follows: [29, 30]

- Failure of validation protocols

- Wrong targets

- False discoveries

- Unstable nature of biomarkers

- No more clinical requirements

- False positivity and false negativity

- Small sample size

- Inadequate controls

In conclusion, cervical cancer is a global gynaecological health issue among only in women which requires more effective and control strategies. The limitations of current screening and diagnostic

strategies for cervical cancer prompt development of molecular biomarkers to improve the clinical outcomes of patients. In order to benefit the patients, basic research requirements are added to clinics. Accurate prediction of treatment response and survival will help to implement personalized therapies that improves treatment response in cervical cancer patients.

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