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Evaluation of a new cervical screening biomarker panel

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Abstract

Primary HPV screening is an emerging concept, which may fundamentally influence cervical cancer screening, making impossible to establish new high throughput automated screening methods in primary screening. Following this path, the combination of HPV testing with screening biomarkers could be the next major milestone. With the advent of a fully automated, low cost, biochemical screening method both the overall quality of the cervical screening programs could be improved and also the programs could be more accessible for women, especially in low resource countries.
We have developed a new gene expression, HPV combination panel for cervical screening. The genes were identified using TaqMan® Custom Array on 293 patient sample, detecting expression of 190 preselected genes in duplicates (the overall number of screening PCR reactions were 114,000). Thorough analysis revealed a classificatory system which identified 8 marker genes, which were capable to produce ROC area better than 0.9 and classify the samples with high precision. The method was validated on a colposcopic referral population using liquid-based cytological specimens, giving 95% specificity and 80% sensitivity for CIN1+ histology.

Materials & Methods

For gene expression studies (ABI1700) cell lines (HeLa, C33A) and CIN3 and cervical carcinoma clinical samples and normal cervical epithelial tissues were purchased. All clinical samples were obtained with medical-ethics approval and all patients gave informed consent. 293 cervix samples taken for routine liquid-based cytology from patients between 18 and 65 years. LBC specimens were transported between 4 and 8 °C before DNA- and RNA isolation. DNA isolated from 5 ml LBC cell suspension using the AmpliLute Liquid Media Extraction Kit (Roche; Cat# 03750540 190) according to the instructions of the manufacturer. RNA isolation from 4 ml LBC samples and for gene expression studies RecoverAll Total Nucleic Acid Isolation Kit (Ambion; Cat#: AM1975) according to the instructions of the manufacturer was used. Reverse transcription was carried out with TaqMan Reverse Transcription Reagents, Kit (Applied Biosystems; Cat#: N808-0234) with random hexamers.
Diagnostic HPV assays were carried out with the following systems: Roche's Amplicor HPV Detection Kit and GenoID's Full Spectrum HPV Amplification and Detection System (FS). For Digene's hC2 HybridCapture 4 ml sample was extracted. Besides these GenoID's HPV Real-Time Assay for ABI7900HT was performed on the extracted DNA samples for clinical evaluation purposes. All assays were performed according to the manufacturer's instructions.
To quantify the cell amount in the samples we determined the genomic DNA copy number with help of a Q-PCR reaction designed specific for a factor V. Real-Time PCR amplifications were made using AmpliTaq Gold DNA polymerase (ABI, HV2860), and StepOnePlus Real-Time PCR System (Applied Biosystems).
384 Wells TaqMan Low Density Arrays real-time PCR amplifications were carried out using 7900HT Fast Real-Time PCR System (Applied Biosystems), TaqMan Universal PCR Master Mix (Applied Biosystems; Cat#: 4324018) on custom-designed 384-wells 7900HT Gene Expression Micro Fluidic Cards (Applied Biosystems) containing primers and probes specific for 1921genes in duplicates (including 18S RNA as house-keeping gene). We selected these 191 genes according to the data of the previous ABI 1700 cDNA microarrays.

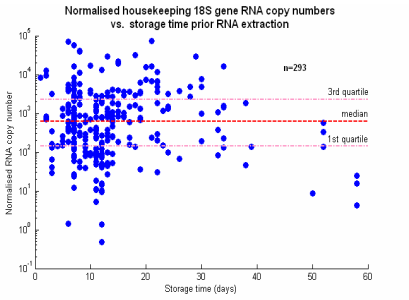

Figure 1. Normalized housekeeping 18S gene RNA copy numbers vs. Storage time prior RNA extraction
Relative 18S RNA copies were calculated on basis of the CT values of TaqMan Low Density Array measurements and plotted against storage time before RNA extraction. Samples were taken in LBC medium and transported at ambient temperature and stored at 4 C°. Symmetrical distribution of values around median indicate no significant deterioration of RNA content over time.

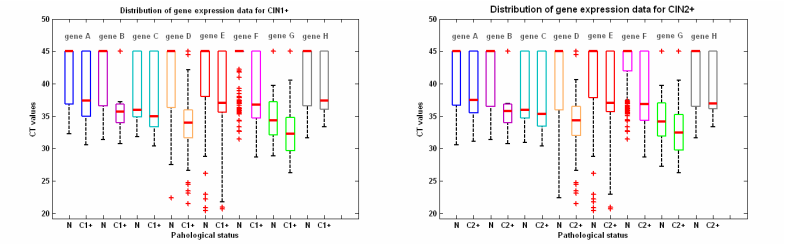

Figure 2. Distribution of gene expression data of normal and different cervical dysplastic cytological samples for geneset1 biomarker genes
Distribution of the eight geneset1 genes expression values were determined using TaqMan Low Density Array measurements (n=293). The members of geneset1 were selected using datamining tools. The distribution of histology confirmed normal and dysplastic samples plotted separately for each gene, the box has lines at the lower quartile, median (red), and upper quartile values. Whiskers cover 1.5 times the interquartile range from the ends of the box. Outliers are displayed with a red + sign. Note that for gene A,B,D,E,F,H the median for the normal samples is no detected expression (CT=45). The modest difference of the gene expression distribution of geneset1 genes between CIN1+ and CIN2+ samples underlines biological similarities of different forms of cervical dysplasia. In both cases gene B and gene F have the highest discrimination power and on the contrary gene C and gene G have very similar distribution.

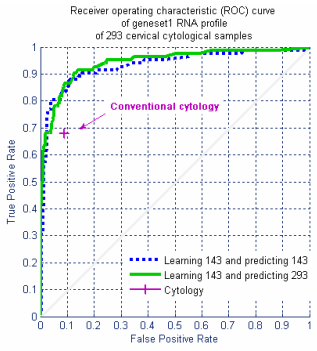

Figure 3. Receiver operator characteristic (ROC) curve of combination HPV and geneset1
A HPV High-risk status (FS) and geneset1 combined classifier is used to predict the pathological status of LBC specimens.

Discussion

The introduction of biomarkers and potentially replacing cervical cytology in its high specificity screening test role could be the next milestone in cervical carcinoma screening. This would open the door for an automated one-step screening test, eliminating subjective, time-consuming elements of the today protocols. On the contrary of the suspected low preanalytical RNA stability, the used specimen collection method showed low deterioration of RNA of the housekeeping 18S gene over time. The benefit of such protocol that it fits with today cytology involving cervical screening protocols and enables easy introduction and parallel testing with the established testing methods. The selected geneset1 biomarker is the same or better than conventional cytology to predict CIN1+ diagnosis of histology in our study using colposcopic referral population.

Acknowledgements

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