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Krüppel Like Factors Family Expression in Cervical Cancer Cells

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Background and Aims. Krüppel Like Factors (KLF) refers to a family of seventeen members of transcription factors. Involved in several cellular processes. As other cancer types, Cervical Cancer (CC) presents molecular deregulations in transcription factors, but especially Human Papilloma Virus (HPV) sequences. Here in this work we analyzed the mRNA expression of all KLF family members in CC-derived cell lines and CC tissues.

Methods. The cell lines used were HeLa, INBL, RoVa, C4-I, Ms751, ViPa, CaLo, SiHa, CaSki, C33a and ViBo and the non-tumorigenic HaCaT. mRNA expression was analyzed by means of expression microarray and RT-PCR, and KLF5 protein by immunofluorescence.

Results. The cell lines were grouped according to HPV genotype as HPV16, HPV18 positive or HPV negative cells. Heterogeneous expression was observed among the cell lines. Despite the heterogeneous expression profile, KLF3, −5, −12, −15 and −16 transcripts were present in all cell lines, KLF4 and −10 which were not expressed in CaSki; KLF11 and 13 were not expressed by Vipa and C4-I, and KLF7 was not expressed by C4-I and Rova. The CC tissue analysis shows expression of most of the KLF members, such as KLF5. KLF5 immunosignal was positive in the three cell lines analyzed.

Conclusions. We suggest that KLF expression could not be related to HPV presence/genotype, at least at transcriptional level, and the expression of KLF family members may be necessary in the biology of the CC cells. © 2017 IMSS. Published by Elsevier Inc.

Key Words: Krüppel Like Factors, Cervical cancer, Cell lines, Microarray profile, Expression, Protein.

Introduction

Cervical cancer (CC) is the second most common cancer in women worldwide. The majority of the cases occur in developing countries where it has been established as an important public health issue, standing among the top leading causes for cancer death among women's. It is raised from previous epithelial lesions ranging from Low-Grade Squamous Intraepithelial Lesions (LSIL) to High-Grade Squamous Intraepithelial Lesions (HSIL) and finally CC (1). CC has several risk factors being the most important etiological factor the presence of Human PapillomaVirus (HPV); more than 90% of the CC contains HPV that is acquired in the majority of the cases through sexual intercourse. In CC as many other cancer types, the transcription factors play an important role in the development and progression of cancer, they play a

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central role in the development of cancer, since several signaling pathways altered in carcinogenic events culminate with the activation of transcription factors and consequent gene expression (2).

Krüppel like factors (KLF's) are a family of DNA-binding transcription regulators that play diverse roles, such as cell proliferation, differentiation, and apoptosis among others during normal development and under several disease conditions. To date, there are 17 family members described and termed KLF1 through KLF17 according to their chronologic description (3). The three highly conserved C2H2 domains at the carboxyl-end characterize them and all members of the family preferentially bind to "GC box" and/or "CACCC" element sites; they can function either as a *trans*-activation or *trans*-repression elements in a cell-type and promoter dependent manner (3). Studies over the past several years support a significant role for this family of transcription factors in carcinogenesis by enhancing or repressing transcription of many genes related to cell cycle such as $p21^{WAF1/CIP1}$, cyclin D1, p27 and p15 amongst others (4).

The characterization of the transcriptome, and in particular the identification of key transcription factors of the several existent CC-CL is crucial for the better understanding of the molecular basis of CC. Therefore in the present work we analyzed the KLF family members mRNA expression by means of microarray expression, RT-PCR in CC-CL and CC expression microarray experiments.

Methods

Cell Culture

The eleven cell lines used were HeLa, SiHa, CaSki, Rova, Vipa, C33a, C4-I, HaCat, Calo, Ms751 and Inbl, they where purchased from the ATCC, except for Vipa, INBL Rova and Calo which where a kind gift from Dr. Alberto Monroy (UIMEO, México). Cell lines were cultured in DMEM medium supplemented with 10% of fetal bovine serum and 1% of penicillin/streptomycin at 37°C in 5% CO_2.

RNA Extraction and Reverse Transcription Reaction

Total RNA was extracted by using the RNAeasy tissue Mini Kit (Qiagen Inc, CA, USA). Cells at 70% of confluence were disrupted and homogenized in 1 ml of Qiazol Lysis Reagent, and then incubated at room temperature for 5 min. After that was added 200 µl of chloroform and incubated at room temperature 3 min. The mixture was centrifuged 12,000 g for 15 min at 4°C. The aqueous phase was transferred to a new tube and mixed with an equal volume of 70% ethanol. The samples were transferred to RNAeasy Column in a 2 mL tube, centrifuged at 8,000 g for 15 s. After centrifugation, was added 700 µl of RW1 buffer and centrifuged at 8,000 g for 15 s. Flow trough was discarded and added 500 µl of RPE buffer to the membrane and then centrifuged 8,000 g for 15 s (2×). The column was transferred to a new collection tube then was added 30 µl of RNAse free water and centrifuged for 1 min at 8,000 g, RNA elution was measured in a Nanodrop-ND-1000 (Thermo Scientific, DE, USA) and RNA integrity was checked in 1.5% agarose gel. After purification, 1 µg of total RNA was used for RT using the High Capacity RNA-to cDNA Master Mix (Applied Biosystems, CA, USA) adding 4 µl of Master Mix, the reaction mixture was incubated at 25°C for 5 min., 42°C for 30 min., 85°C for 5 min., according to manufacturers protocols.

mRNA Differentially Expressed in CC Tissue Analysis

Microarray hybridization, scanning and analysis. For this approach 4 CC and 2 normal cervical tissues were used, and the cervical cancer cell lines previously mentioned. The microarray used for these studies was Affymetrix GeneChip 1.0, which contains over 750,000 probe sets representing all of ~28,800 annotated genes. Sample amplification and preparation for microarray hybridization was performed according to Affymetrix specifications (http://media.affymetrix.com/support/downloads/manuals/wt_expressionkit_man ual.pdf). In brief, 100 ng total RNA was reverse transcribed to cDNA, amplified by in vitro transcription and reverse transcribed to cDNA again. Fragments between 40 and 70 bp were generated enzymatically, labeled and hybridized onto the microarray chips in an Affymetrix hybridization oven at 60 rpms, 45°C for 17 hours. Chips were washed according to the established protocols (Affymetrix, Santa Clara, CA, USA) with GeneChip fluidics station 450, and finally they were scanned with an Affymetrix 7G GeneChip scanner.

Microarray analysis was achieved by means of CEL files of the Partek Genomics Suite 6.5v software (Partek Incorporated, Saint Louis, MO) and Transcriptome Analysis Console (Affymetrix, Santa Clara, CA, USA). Probe sets were summarized by means of Median Polish and normalized by quantiles with no probe sets excluded from analysis. Background noise correction was achieved by means of Robust Multi-chip Average (RMA) and data were log2- transformed. Data grouping and categorization was achieved by principal component analysis (PCA). Differentially expressed genes were detected by means of ANOVA. Genes were considered altered with +1.5 or −1.5 fold change. Also less astringent analysis was carried out, using ±1 fold change to identify mRNA molecules whose variation was little, but still expressed. GEO accession number to raw CEL files is GSE89657.

KLF's Transcripts Detection by PCR

Primers for each-specific gene was in-house designed using Primer-BLAST software in the NCBI website with the NCBI reported sequences and reported previously (5), twelve µl of GoTaq® Green Master Mix (Promega, WI, USA), 500 ng of cDNA template of each cell line, 20 pmol