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Epigenetic Regulation of Hematological and Neurological Expressed 1(HN-1) at DU145 Brain Metastases Prostate Cancer Cell Line

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Abstract

Epigenetic investigates gene expression modifications not due to DNA sequence alterations. Gene expression modifications appear with packing of DNA with variety of chromatin structures. The most studied forms of epigenetic phenomenon are DNA methylation and histone modifications. These pathways are connected with each other and reversible. DNA methylation reported genes: 36B4 (as control), HN1 primers were used and methylation status had been confirmed at DU145 cell line. Histone modifications which close the gene expression is histone deacetylation had been reversed by Trichostatin A (TSA). It is reported that inhibition of methylation and deacetylation in a serial way result with successful gene activation, performed with 5 Azacitidine and TSA incubation at the project. In the recent years, epigenetic base had been shown for many diseases that research and development for epigenetic disorders won successful treatment cases. Preclinical and clinic levels of candidate drugs are on the way.

Keywords: HN1; DU145; TSA; 5-Azasitid

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Abbreviations

ARE: Androgen Response Element; AZA: Azasitidin; HN1: Hematological and Neurological Expressed 1; TSA: Trikostatin A

Introduction

Cancer is a kind of disease which incidence has been increasing depending on the changing conditions of life and poses a grave threat to modern societies. Cancer which is characterized by uncontrolled cell growth and spread of abnormal cell structures to the organism is an epigenetic disease at the same level that it can be considered a genetic disease [1-3]. Therefore, cancer research has focused on epigenetic mechanisms that play critical role on carcinogenesis for the last decade. Within the scope of the project, a variety of gene expression changes were searched following demethylation [4] and inhibition of deacetylation [5] in prostate cancer cell line DU145 [6]. Also it was investigated whether there is a cross talk between the methylation and acetylation pathways after using the same demethylation agent with HDACI-Trikostatin A as a combination. Hematological and neurological expressed 1 (HN1) mRNA was first identified in the tissues of mouse embryos [7] and has also been detected in various other tissues. The gene is highly conserved in vertebrates [8] (although not in invertebrates), suggesting that HN1 has a conserved function in the development of the tissues in which is expressed. Furthermore, several studies have suggested that HN1 expression is associated with metastatic cancer progression [9] and neural development [10], as well as with nerve and retina regeneration [11]. Recently, Laughlin demonstrated that HN1 is involved in the malignant phenotypes of brain tumors [12] HN1 is a ubiquitously expressed, EGF-regulated gene. Expression of HN1 in prostate cell lines down-regulates PI3 K- dependent Akt activation [13]. HN1 is regulated by androgens through the putative androgen response elements (AREs) found in its promoter [14].

Materials and Methods

Cell culture

DU145 cells were cultured in DMEM-Ham'sF12 (GIBCO) with 5% fetal bovine serum, 1%

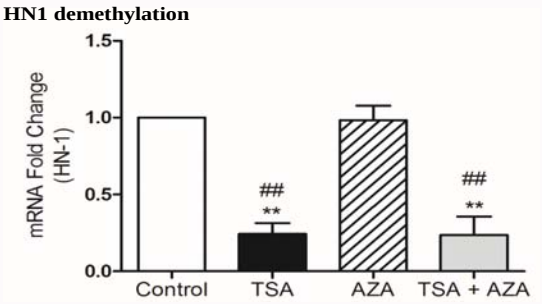

Figure 1: Effect of TSA and 5-Azasitidin on HN1 Expression at DU145 Cells (N=3).

Table 1: Real-Time PCR primers.

GCCAGCGAAGCCACGCTGCTGAAC	36B4 forward
CGAACACCTGCTGGATGACCAGCCC	36B4 reverse
CTAACCCAAGACGAGGC	HN1forward
AAAGGCACAAGAGGCCCTAGAT	HN1reverse

L-glutamine and 1 U/ml of each penicillin/ streptomycin. Cells were incubated at 37°C and 5% CO_2 in a humidified atmosphere.

RNA isolation

Qiagen R Neasy Mini Kit was used.

Epigenetic regulation

Histone deacetylase inhibition by Trichostatin A with 100 nM for each plate and demethylation by 5-Azasitidin with 100 mg/ml for each plate was used and overnight incubation were done.

Real-time PCR

Epigenetic genes primer couples for RT-PCR (Table 1) Real-time PCR was performed using a Light Cycler® 480 (Roche Diagnostics) instrument and Light Cycler 480 SYBR Green 1 Master (Roche Diagnostics) kit. Briefly, reactions were performed in a 20 µl volume with 5 pmol of each primer and 1 µl of cDNA template derived from reverse-transcribed RNA of untreated control and incubated cells. 36B4 housekeeping gene was used as an endogenous control and reference gene for relative quantifications. The same thermal profile was optimized for all primers: a preincubation for 5 min at 950C for 1 cycle, followed by 40 amplification cycles of denaturation at 95°C for 10 sec, a primer annealing at 64C for 20 sec, and a primer extension at 72°C for 10 sec. Water was included as a no-template control. Melting curves were derived aier 40 cycles by a denaturation step at 95°C for 10s, followed by annealing at 65°C for 15 sec, and a temperature rise to 95°C with a heating rate of 0.1°C/s and continuous fluorescence measurement. A final cooling was performed at 37°C for 30 sec. Melting curve analyses of each sample were performed using Light Cycler 480 software version LCS480 (Roche Diagnostics). The analysis step of relative quantification was a fully automated process accomplished by the soiware, with the efficiency set at 2 and the cDNA of untreated cells defined as calibrator. HN1 primers were amplified using (10 pmol of each) primers which were designed using Light Cycler Probe Design 6oiware 2 (Roche, Germany).

Statistical analysis

All the illustrated results represent one of at least three independent experiments with similar outcomes.

Results

Epigenetic pathway was searched at DU145 cells. HDAC Inhibition by Trichostatin A and demethylation by 5-Azasitidin had been performed and HN1 specific primers were used at RT-PCR (Figure 1).

$^{**}P \leq 0.01$ Versus Control, $^{\#\#}P \leq 0.01$ Versus AZA; Bonferroni Test After Single Sided Anova Test.

TSA caused decrease versus control. ($^{**}P \leq 0.01$) and demethylation with AZA respond as control and usage of both reagents decrease as TSA ($^{\#\#}P \leq 0.01$).

Discussion

Project was designed as to mimic severe carcinogenesis of prostate. DU145 is the brain metastases of prostate cancer reached to the target. Control group with closed histone showed HN1expression and inhibition of deacetylases with TSA, HN1 expression was decreased. As third group demethylation with 5-azasitidin also showed the expression level as control and it presents the homogeny expression distribution panel of HN1 [14]. Both reagents repeat the abundant role of TSA. The epigenetic regulation of HN1 had been shown for the first time in this report.

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