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Activation of some signalling pathways in Rat-1 cells induced by Epstein-Barr virus LMP1 gene with mutations in HOS recognition sites

S. Diduk[1], K. Smirnova[1], V. Gurtsevitch[1]
[1]*Cancer Research Center, Laboratory of Viral Carcinogenesis, Moscow, Russian Federation*

Introduction. Epstein-Barr virus (EBV) transforms human B lymphocytes into indefinitely proliferating lymphoblastoid cell lines in vitro and is associated with several human malignancies in vivo. Latent membrane protein 1 (LMP1) is essential for EBV-mediated lymphocyte transformation. Two sites of LMP1 molecule, CTAR1 and CTAR2, are pivotal for activation of numerous signal transduction pathways and cell transformation. More recently it has been found that CTARs regions of LMP1B95-8 prototype variant contains one canonical and one cryptic HOS recognition site. The major mutations within these sites abrogated HOS binding and increased transforming activity of LMP1. The aim of our study was to investigate the influence of the major mutations (G212S/S350A/S366T) in canonical and cryptic HOS recognition sites within CTAR1 and CTAR2 of LMP1 molecule on activation of NF-kB and AP1 signal pathways and on intracellular generation of reactive nitrogen species (RNS).

Material and methods. Plasmids. pBabe-puromycin, pSG5-LMP1B95-8 and LMP1Cao, pSG5-G212S, S350A, S366T, G212S/S350A, G212S/S366T, Triple (G212S/S350A/S366T), pkB-ConA-Luc, pAP1-Luc, cell lines HEK293, Rat1, Nitrate/nitrite assay kits (Cayman,USA). Transformation and transduction of cells, western blotting, nitrate/assay, anti-LMP1 antibody (S12), anti-mouse antibody (Sigma).

Results. The data obtained demonstrated that the transforming activity of wild-type LMP1B95-8 with double substitutions (G212S/S350A or G212S/S366T), as well as triple amino acid substitutions G212S/S350A/S366T (Triple), was significantly higher than prototype LMP1B95-8 variant and comparable with highly tumorogenic LMP1Cao. LMP1Cao and LMP1Triple had limited binding to the E3 ubiquitin ligase as well as slightly enhanced NF-kB activity, but they hadn't any influence at AP-1 pathway activation. Since RNS can regulate the activity of many transcription factors including NF-kB, AP-1 and some others, we investigated generation of NO in Rat-1 cells transduced with mutated variants of LMP1. Our results show that LMP1B95-8 and LMP1 proteins with single substitutions (G212S, S350A and S366T) produced similar levels of NO, but much higher than Triple and Cao variants.

Conclusions. Mutations in HOS recognition sites of LMP1B95-8 abrogated its ability to inhibit NF-kB and AP1 activation and decreased RNS generation by LMP1Cao and Triple that correlated with its cell transforming capacity This work was supported by the Russian FFI 07-04-00604.

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Stearoyl-CoA desaturase promotes proliferation of prostate cancer cells via induction of lipogenic gene expression

E. Kim[1], S. Kim[1], H. Choi[1], O.J. Kim[1], S.S. Park[1]
[1]*Chonnam National University, Biological Sciences, Gwangju, Korea*

Background: Stearoyl-CoA desaturase (SCD) deficiency shows reduced expression of various lipogenic genes in the SCD knock-out mouse liver. Since altered expression of lipogenic enzymes has been known to play an important role in prostate cancer development, we hypothesize that SCD may promote proliferation of prostate cancer cells via upregulation of lipogenic gene expression.

Materials and Methods: We generated SCD overexpressed or suppressed LNCaP cells, a human androgen-dependent prostate cancer cell, by stable transfection with SCD expression or SCD miRNA vector. Cell proliferation and cell death rates were determined with MTT and trypan blue dye exclusion assays, respectively. CleavaLite Caspase-3 activty assay was used for caspase-3 activity after 24h treatment of ceramide (20 μM). To assess SCD effects on the expression of lipogenic genes, semi-quantitative PCR was used.

Results: SCD promotes cell proliferation of androgen-dependent LNCaP cells. Furthermore, SCD overexpressed LNCaP cells was resistant to ceramide-mediated apoptosis via suppression of caspase-3 activity. In contrast, SCD-mediated induction of LNCaP cell proliferation was abolished by suppression of SCD expression. SCD induced expression of fatty acid synthase (FAS) and acetyl-CoA-carboxylase α (ACCα). In contrast, suppression of SCD expression inhibited expression of these lipogenic enzymes in LNCaP cells, suggesting that SCD-mediated induction of LNCaP cell proliferation may go through induction of FAS and ACCα expression. Furthermore, cell growth promoting effect of SCD was completely abolished in SCD overexpressed LNCaP cells after treatment of cerulrenin, a specific inhibitor for FAS.

Conclusions: SCD overexpression induced proliferation of LNCaP cells and protected the cell death from the apoptotic stimuli. Together, our data shows that SCD promotes proliferation of prostate cancer cells through increased expression of FAS and ACCα genes, demonstrating important roles of SCD in prostate cancer progression.

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Crosstalk between estrogen and insulin signaling systems in breast cancer cell lines

M.M. Dias[1], G.Z. Rocha[1], J.C. Oliveira[1], J.B.C. Carvalheira[1]
[1]*UNICAMP, Internal Medicine, Campinas, Brazil*

Background: Obesity has been consistently shown to increase rates of breast cancer in postmenopausal women. The hormonal changes associated with obesity are considered to be responsible for these adverse effects, with particular emphasis being placed on the increased production of peptide and steroid hormones, such as oestrogens and insulin. Objective: In this study, we investigated the possibility of direct interactions between insulin and 17β-estradiol (E2) action in the breast cancer MCF-7 and ZR75 cell lines, focusing on some key intermediate steps in the PI3K/Akt/mTOR signaling pathway. Methods: Western blotting and MTT cell proliferation assays were conducted on MCF-7 and ZR75 cells to evaluate the crosstalk between insulin and estrogen signaling pathways. Results: Our data show that insulin and E2 alone were able to increase proliferation of the breast cancer cells and to produce molecular activation of the Akt/mTOR signaling pathway. However, combined administration of insulin and E2 not only led to a significant increase in MCF-7 and ZR75 proliferation, which was abrogated by rapamycin administration, but also provoked a quantitative potentialization of molecular signaling through the Akt/mTOR pathway. Conclusion: We provide evidence for a direct and positive crosstalk between insulin and estrogen signaling at the level of Akt/mTOR pathway in MCF-7 and ZR75 breast cancer cells. This mechanism may serve to potentiate the activity of both the insulin and estrogen pathways and to increase stimulation in physiological processes, such as cell growth and proliferation.

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Investigation of the EMSY gene and protein in patients with ovarian cancer

N. Buyru[1], J. Altinisik[1], T. Ulutin[1]
[1]*Cerrahpasa Medical Faculty, Medical Biology, Istanbul, Turkey*

Background: Ovarian cancer is the fourth most common malignancy in women and is the leading cause of death from gynaecological cancers. Inherited mutations in either BRCA1 or BRCA2 genes account for approximately 75 % of familial breast-ovarian cancer. However the virtual absence of BRCA1 and BRCA2 mutations in sporadic breast and ovarian cancers is unexplained.

The EMSY gene, mapped to 11q13.5 has been found to be amplified in sporadic breast and ovarian cancer with a frequency of %13 and %17, respectively. EMSY codes for a protein of 1322 amino acids which binds to exon 3 of the BRCA2 gene and suppresses the activation of BRCA2-GAL4 complex. In sporadic breast and ovarian cancer cases the EMSY gene is amplified and excessive EMSY protein is synthesized. This excess protein interacts with BRCA2 and prevents it from repairing damaged DNA.

Material and methods: Tumor samples of 50 patients with sporadic ovarian cancer and 17 benign ovarian tumors were enrolled in our study. The EMSY gene overexpression and the amount of the EMSY protein were investigated by Real Time PCR and Western Blotting, respectively.

Results: EMSY overexpression and increased EMSY protein were detected in 6 (%12) of 50 patients. When evaluated with respect to the histological types of the tumors the frequency of overexpression of the EMSY gene was found 12.5 % and 14.3% in serous and mucinous types of epithelial ovarian tumors, respectively.

Conlusion: Overexpression of the EMSY gene is observed in a subset of patients with sporadic ovarian cancer which may indicate repression of the BRCA pathway in these sporadic cases.

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Differential expression of PPARgamma1 and gamma2, and ERalpha in MCF-7 and MDA-MB-231 breast cancer cell lines

R. Nasir[1], N. Mohd. Nor[2], N.S. Yaacob[1]
[1]*Universiti Sains Malaysia, Chemical Pathology, Kota Bharu, Malaysia;*
[2] *Universiti Sains Malaysia, School of Health Sciences, Kota Bharu, Malaysia*

A ligand-dependent nuclear receptor, peroxisome proliferator-activated receptor gamma (PPARγ) has been reported to be expressed in various cancer cells including breast, prostate, colorectal and cervical cancer. Bidirectional signal cross talk has recently been reported to exist between