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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

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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

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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

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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

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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

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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

PPARγ and estrogen receptor (ER) in breast cancer cells. The present study was carried out to evaluate the transcriptional activity of PPARγ isoforms, PPARγ1 and PPARγ2, and ERα in human breast cancer cell lines treated with ligands for PPARγ and ERα, 15deoxy-prostaglandin J_2 (15d-PGJ_2) and 17β-estradiol (E2) respectively, by quantitative Real-Time PCR using homologous internal standards. ER-positive (MCF-7) and ER-negative (MDA-MB-231) breast cancer cells were treated with EC_{50} doses of 15d-PGJ_2 and 10 nM E2 alone or in combination for up to 48 hrs. The ERα, PPARγ1 and PPARγ2 mRNA expression levels were significantly up regulated ($p < 0.05$) in MCF-7 cells treated with 15d-PGJ_2 or E2 alone. The combined treatment of 15d-PGJ_2 and E2 however, significantly down regulated the ERα mRNA expression, showed no significant difference in PPARγ1 mRNA expression and up regulated the PPARγ2 mRNA expression level in MCF-7 cells. The PPARγ1 mRNA expression was significantly up regulated in MDA-MB-231 cells treated with 15d-PGJ_2 alone and in combination with E2. In contrast, no significant difference in the PPARγ1 mRNA expression level was observed in E2 treated cells. The mRNA expression of PPARγ2 was significantly down regulated in MDA-MB-231 cells treated with 15d-PGJ_2 but not with E2 treatment. Interestingly, a significant up regulation of PPARγ2 mRNA expression was observed in these cells when treated with the combination of 15d-PGJ_2 and E2. The differential expression of PPARγ1, PPARγ2 and ERα in ER-positive and ER-negative breast cancer cells suggests a complex regulation of these transcription factors in breast carcinogenesis, the mechanism of which remains to be elucidated.

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Inactivation of the FHIT gene in clear cell renal carcinomas

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FHIT is a tumour suppressor gene which is frequently inactivated in different types of cancer. Yet little is known about the mechanism of FHIT inactivation in clear cell renal carcinomas. Since genetic alterations were not frequently observed in DNA corresponding to the FHIT gene in renal tumours, to elucidate the mechanism of FHIT gene silencing we examined 22 paired samples of clear cell renal carcinoma and non-malignant renal tissue for the methylation of the FHIT 5'CpG island by methylation-specific PCR. Hypermethylation of the FHIT 5'CpG island was detected in 54.5% of clear cell renal carcinomas. Bisulfite sequencing of the FHIT 5'CpG island confirmed the results obtained by methylation-specific PCR for selected samples. We showed that expression of the FHIT gene is inversely correlated with hypermethylation of the FHIT 5'CpG island in the selected samples. Our results suggest that hypermethylation of the FHIT 5'CpG island may be responsible for inactivation of the FHIT gene in clear cell renal carcinomas.

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Profile of methylaion of tumour related genes in breast cancer in Tunisian women

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Background: It is becoming increasingly recognized that aberrant hypermethylation of gene promoter regions is an important mechanism inducing transcriptional silencing of tumor suppressor genes in various human cancer including breast carcinomas. There are several reports on methylation profiles of breast cancer patients from Western population. However, to our knowledge there is no study in Arabian populations till date. It is important to note that Tunisia belong to low incidence zone of breast carcinoma with standardized incidence of 19.6 per 100 000 women. The present study was undertaken to evaluate the DNA methylation profile of tumor-related genes in Tunisian breast carcinomas.

Methods: One hundred and nine invasive ductal carcinomas diagnosed at the Department of Pathology at Farhat-Hached Hospital of Sousse (Tunisia) were investigated for the methylation status of a panel of fifteen known tumor-suppressor and -related genes by methylation-specific polymerase chain reaction. Both specific methylated and unmethylated primers were used for PCR and the products were visualized with agarose gel electrophoresis.

Results: Of the 109 cases 23 (21%) showed methylation at 1 to 3 genes, 36 (33%) were methylated at 4 to 6 genes, and 50 (46%) were methylated in more than 6 genes. No cases were methylated at all fifteen genes and all cases showed at least one gene methylated. Hypermethylation frequencies were 78% for RASSF1A, 66% for SHP1, 61% for HIN1 and BRCA1, 47% for P16 and ER, 42% for CDH1 and APC, 40% for BLU, 35% for DAPK, 34% for RARβ2, 27% for GSTP1, 17% for TIMP3, 14% for CCND2, and 8% for hMLH1.

Conclusion: This study shows high frequencies of methylation of tumor-suppressor and -related genes in Tunisian women in comparison with Western women. These observations suggested that gene hypermethylation may be affected by ethnicity. Besides ethnicity, these epigenetic variations may also be attributed to differences in the risk factors such as life style and dietary habits. Thus, our study underscores the limitation of extrapolation of the Western data to other populations. Our findings, reported here will hopefully provide a stimulus for additional studies comparing populations with different ethnicity and risk factors.

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Do PIKE, PIK3CA and PTEN genes in Phosphoinositide-3-kinase/Akt signaling pathway play a crucial role in progression of high-grade gliomas?

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Background: High-grade gliomas are the most common primary brain tumors and associated with poor survival. Phosphoinositide 3-kinase/AKT signaling pathway is important in the development of malignant gliomas. The PIK3CA gene, encodes the p110alpha catalytic subunit of PI3K, is activated in various cancers. PIKE (CENTG1), encodes a protein that binds to phosphorylated Akt and increases its activity, is frequently amplified in glioblastomas. phosphatase and tensin homology deleted on chromosome 10 (PTEN) is an important regulator of the PI3K/Akt pathway via its ability to antagonize PI3K. PTEN function is lost in high-grade glioma due to loss of heterozygosity or mutations and loss of this gene function associated with activated AKT levels. In this study we aimed to identify the roles of the genes which were components of the PI3K/Akt signaling pathway and correlation between their expression profiles in malignant disease progression.

Materials and methods: Human brain tumor samples were obtained from patients who underwent primary therapeutic subtotal or total tumor resection performed under surgical operation. All cases signed a written informed constant statement approved by local ethics committee. Explant cell cultures were performed from brain tumor tissues of 18 (6 female, 12 male; average age 49.72±14.83) cases. Malignant lesions have been described in the medical history of cases: anaplastic oligoastrocytoma WHO grade III (7 cases), GBM WHO Grade IV (7 cases) and brain metastasis from lung cancer (4 cases). Total RNA was isolated from tumor cells. RNA of the tumor samples were reverse-transcribed with oligo dT primers and quantified by real-time reverse transcription polymerase chain reaction (RT-PCR) performed with the LightCycler instrument. U87MG glioblastoma cell line was used as positive control.

Results: The mean relative ratios of PIK3CA, PIKE and PTEN genes were found; 162.46, 13.67 and 3733.61, respectively. There was no significant association between tumor grades/age and gene expressions. The correlation between PIKE and PTEN gene expressions was found significant especially in anaplastic oligoastrocytoma ($p<0.0001$). Similar correlation was found between PIK3CA and PIKE genes in cases with brain metastasis from lung cancer ($p=0.037$).

Conclusion: Due to these expression correlations, PI3K/Akt signaling pathway genes could be used as pivotal biomarkers and build smart and effective drug combinations of molecular targeted treatments of malignant gliomas.

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Cyclooxygenase-2 dependent regulation of E-cadherin through the transcription repressors Snail and ZEB1 is limited to conventional gastric cancers cell lines

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Background: Approximately 10% of patients present with gastric cancer before the age of 45, so-called early onset gastric cancer (EOGC), and it is postulated that genetic factors may play a more important role than in conventional gastric cancer (presenting > 45 years old). EOGCs have been shown to have a different molecular pathway than conventional gastric cancers and we have shown previously that they have a strikingly low expression of COX-2 compared to conventional gastric cancer, where it is often overexpressed.

Aims: COX-2 regulation of E-Cadherin has been shown to occur in lung cancer and given that E-Cadherin is critical in gastric carcinogenesis, we examine the relationship between these two molecules in this study. In