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Comparative analysis of adaptor protein CIN85/Ruk isoforms expression in human benign prostate hyperplasia and prostate adenocarcinoma

O.I. Basaraba[1]*, Ya.P. Bobak[2], G.Yu. Shuvayeva[2], V.L. Buchman[3], L.B. Drobot[1]. [1]*Palladin Institute of Biochemistry of the National, Laboratory of Cell Signalling, Kyiv, Ukraine,* [2]*Institute of Cell Biology, Deparnnent of Cell Signaling, Lviv, Ukraine,* [3]*Cardiff University, Neuroscience Group, School of Biosciences, Wales, UK*

Adaptor proteins play an important role in facilitating protein-protein interactions and subsequent formation of signalling networks. These proteins recruit binding partners to a specific location inside the cell, and also regulate their activity. Adaptor protein Ruk/CIN85 is important components of different regulatory pathways involved in control of cell proliferation, adhesion, invasion and survival, and, thus, an play a role in uterine carcinogenesis. Neoplastic transformation of prostate tissue includes a broad set of oncological diseases, but the molecular mechanisms of these pathological processes remain unclear.

The main goal of our research was to study cin85/ruk gene expression in samples of human benign prostate hyperplasia (BPH) and prostate adenocarcinoma both at the level of mRNA and protein using Northern blot and Western blot analysis.

Using Northern blot analysis, one cin85/ruk mRNA transcript of approximately 3.2 kb was detected in analyzed samples of prostate tumors. This mRNA transcript codes for full-length form of adapter protein CIN85/Ruk with molecular weight of 85 kDa. Both BPH and adenocarcinoma samples were characterized by polymorphism in the expression level of 3.2 kb mRNA transcript of cin85/ruk. Using anti-RukS Western-blot analysis, multiple molecular forms of CIN85/Ruk with molecular weights of 140, 130, 100, 85 and 50 kDa were detected in the samples of BPH. Pattern of multiple molecular forms of CIN85/Ruk in prostate adenocarcinoma samples differed from the previous ones by appearance of the additional immunoreactive bands corresponding to p70, p56, p40 and p34. Our data suggest that the pattern of Ruk/CIN85 expression depends on specific molecular features characteristic for individual samples.

The obtained results suggest that changes in the expression level of cin85/ruk mRNA transcripts and multiple molecular forms of CIN85/Ruk in BPH and adenocarcinoma samples can lead to the loss of coordinated control of apoptosis and proliferation in the transformed cells. These data will offer new opportunities for the identification and validation of key molecular tumor targets to be exploited for novel therapeutic approaches.

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Is obesity changing the expression profile of genes coding IGF in the colorectal cancer patients?

M. Muc-Wierzgon[1]*, E. Nowakowska-Zajdel[1], R. Braczkowski[2], T. Kokot[1], U. Mazurek[3], M. Rudzki[4], D. Waniczek[4]. [1]*Medical University of Silesia, Department of Internal Medicine, Bytom, Poland,* [2]*Medical University of Silesia, Department of Public Health, Bytom, Poland,* [3]*Medical University of Silesia, Department of Molecular Biology, Sosnowiec, Poland,* [4]*Medical University of Silesia, Department of Surgery, Bytom, Poland*

Epidemiological researches indicate that obesity is a risk factor of colorectal cancer. The fatty tissue is a place of synthesis and excretion of many cytokines as IGF1, TNFα, IL-6, VEGF, TGFβ, leptin, adiponectin and others.

The aim of the study was to analyse mRNA expression profile of genes coding IGF in relation to body mass index (BMI) in the colorectal cancer patients.

Material and Methods: The colon cancer specimens were taken during surgery treatment of 35 colorectal cancer patients (22 men; 13 women, aged 65.5$\pm$8.9). Examined patients were divided into I–IV groups according to TNM Classification (I-7, II-9, III-10, IV-9 patients). They were divided into A and B groups as well, in relation to BMI (A: BMI $<$ 25) – 17 patients, B: BMI $\geqslant$ 25) – 18 patients). A number of mRNA copies of genes coding IGF1, IGF2, IGF1R and IGF2R were examined with QRT-PCR method. The experiments were performed according to the protocol approved by the Ethic Committee of the Medical University of Silesia in Katowice.

Results: There were no statistical differences of mRNA copies of genes coding IGF1, IGF1R, IGF2, IGF2R in the tumor tissue between examined groups A and B. But the analysis showed that the number copies of mRNA IGF1 was enough higher in the patients with overweight and obesity than in the group with normal weight (15052$\pm$4077 versus 8558$\pm$2409). The level of mRNA genes coding IGF were similar in tissues representing different clinical staging but the analysis showed the higher number copies of IGF1, IGF1R, IGF2 according to advancement of cancer. There were no correlation between the number of mRNA copies of genes coding IGF and BMI in the group A. But there was negative correlation between the number of IGF1 and BMI ($R = 0.6727$; $p = 0.330$) and positive correlation between the number of IGF2R and BMI ($R = 0.5441$; $p = 0.0238$) in the group B.

Conclusions:

1. The changes of expression profile of genes IGF and its receptors in colorectal cancer patients according to body mass were found.
2. The analysis should be done among patients according to level of advancement of cancer disease, taking into consideration body mass index for the same clinical staging.
3. The findings suggest that the changes of expression profile of genes coding IGF could be connected with autocrine and paracrine function of tumor cells in colorectal cancer.

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"AminoIndex" for cancer detection (2): plasma free amino acid profiling for breast cancer screening

H. Yamamoto[1]*, N. Okamoto[2], A. Chiba[3], Y. Miyagi[2], A. Imaizumi[4], T. Ando[4], M. Onuma[1], M. Yamakado[5], A. Yoshida[3]. [1]*Ajinomoto Co., Inc., HI (Health Informatics) Department, Tokyo, Japan,* [2]*Kanagawa Cancer Center, Cancer Prevention and Cancer Control Division, Yokohama, Japan,* [3]*Kanagawa Cancer Center, Department of Breast and Thyroid Surgery, Yokohama, Japan,* [4]*Ajinomoto Co., Inc., Institute of Life Sciences, Kawasaki, Japan,* [5]*Mitsui Memorial Hospital, Center for Multiphasic Health Testing and Services Tokyo, Japan*

Introduction: Amino acids balance is changed in patients of various diseases due to metabolic transition while it is maintained in healthy human such as various cancers. We previously demonstrated that significant changes of plasma amino acid profile was observed and classifier composed of plasma amino acid concentrations as explanatory variables ("AminoIndex") showed high discrimination ability for breast cancer patients (Okamoto 2009). In this study, further possibilities of "AminoIndex" for breast cancer were investigated.

Subjects and Methods: Venous blood samples were collected from Japanese breast cancer patients before any medical treatment (N = 109). Those of controls were also collected from subjects who were undergone comprehensive medical examination at Mitsui Memorial Hospital (N = 1,699). After plasma separation, amino acid concentrations were measured by LC-MS.

60 patients and 300 age-matched control subjects were chosen as study data set to predict "AminoIndex". And

the rest were used as test data set to valid the predicted "AminoIndex".

Results: Plasma concentrations of several amino acids were significantly changed in breast cancer patients compared to control subjects in study data set. Finally, "AminoIndex" for breast cancer composed with six amino acids (Gln, Ala, ABA, Trp, Orn, and Arg) was predicted.

To evaluate the performance, the ROC curve was calculated, and this gave an AUC of ROC of 0.832 using the study data. Validation of predicted "AminoIndex" using test data set resulted same discriminating performance (AUC of ROC of 0.822), suggesting the robustness of the predicted classifier.

Furthermore, predicted "AminoIndex" showed notably features.

1. The index could discriminate breast cancer patients in early stages.
2. The index showed higher discrimination performance than those of existing tumor markers especially in stages 0, I, and II patients.
3. The index could equally discriminate breast cancer patients of any histological types.

Therefore, predicted "AminoIndex" would be suitable for screening and early detection of breast cancer patients.

Conclusion and Perspectives: In this study, we demonstrated that change of plasma amino acid profile would be a helpful tool for early detection of breast cancer patients. "AminoIndex" would be useful to concentrate and inspire the candidates for further survey such as mammography. To evaluate the efficacy of this method, cohort studies are ongoing.

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p63, K14 and p53 expression in epithelial layers of tumor-distant oral mucosa in patients with oral squamous cell carcinoma

S. Kirieieva[1]*, N. Yurchenko[1], M. Sidorenko[1], V. Protsyk[2].
[1]*Institute of Cryobiology and Cryomedicine of Natl Ac Sci of Ukraine, Department of biotechnical Problems of Diagnostics, Kyiv, Ukraine,* [2]*National Institute of Cancer, Kyiv, Ukraine*

The p63 gene is highly expressed in the epithelial stem cells and progenitor cells of epithelial tissues and SCC. p63 plays an essential role in epithelial development, stem cell identity and cellular differentiation, and maintenance of proliferative potential of basal keratinocytes. K14 is major keratins of basal cells. Both protein markers p63, K14 expressed in cells of basal layer of epithelium of normal mucosa. p53 gene is the most altered gene in premalignant lesions and OSCC.

Aim: Investigation of the expression of p63, K14 and p53 in tumor-distant oral mucosa from patients with OSCC.

Material and Methods: Biopsy specimens of tumor-distant mucosa and tumors were obtained from 18 patients with OSCC. Most of the patients have the smoking-drinking status and professional contact with carcinogen. The section of tumor-distant mucosa and tumors were classified according to the UICC. Tissue sections were immunohistochemically stained using monoclonal antibodies: for p63 (clone A4A), for K14 (clone 124), for p53 (clone DO-7), "Daco", counted according to ephitelial layers as labeling index (LI, %).

Results: In tumor-distant oral mucosa revealed the progression of histopathological phenotype: from hyperplasia to high dysplasia, Ca in citu and OSCC, I degree. The analysis showed the architectural alteration of cells p63 and K14 expression in the epithelial layers of tumor-distant mucosa and distribution in suprabasal layers. The suprabasal p63, C14 and p53 expression is strongly associated with grade dysplasia and development of OSCC. IL of p63 and C14 decrease in basal layer and increase in suprabasal layers from mild dysplasia to high dysplasia, carcinoma in situ and OSCC (range, LI, p63, C14, mild dysplasia, 1/bas, 45.0–56.0, l/supr 29.0–31.0; 1/bas 24.0ndash;35.5, l/supr 16.3–27.1; high dysplasia, l/bas 11.6–23.0, l/supr 34.6–70.0; 1/bas 13.0–21.5, 1/supr 30.0–34.5, respectively). In carcinomas in tumor-distant mucosa and in primary OSCCs the p63 and K14 cells represented essential part of tumor cells population of OSCC (range, LI, p63, K14, carcinoma in situ and OSCC in tumor-distant mucosa, 15.0–45.6; 10.0–51.3, respectively; primary OSCC, 19.7–79.1; 8.4–54.3, respectively). It's proposed precipitation of stem/progenitor cells in development of transformation phenotype in oral mucosa and in biology of OSCC.

Conclusion: Immunohistochemically revelation of cells p63, K14 and p53 expression in surface suprabasal layers of oral mucosa have been used to objectify differential diagnostic and monitoring the risk after treatment for cancer prevention.

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Mutagen sensitivity and p53 in suprabasal layers of tumor-distant oral mucosa in patients with oral squamous cell carcinoma

S. Kirieieva[1]*. [1]*Institute of Cryobiology and Cryomedicine of Natl Ac Sci of Ukraine, Department of biotechnical Problems of Diagnostics, Kyiv, Ukraine*

Background: The epidemiology associates OSCC with long-term smoking tobacco, being alcohol, exposure to various carcinogens and genetic predisposition. The p53-tumor-supressor gene is the most altered gene in OSCC.

Aim: Investigation of the sensitivity of lymphocytes to mutagen bleomycin and p53 immunoexpression in tumor-distant oral mucosa and tumor in patients with OSCC.

Material and Methods: Peripheral blood lymphocytes and biopsy specimens of tumor-distant oral mucosa and tumors were obtained from 18 patients with OSCC (male, age from 43 to 79 years). Most of the patients have the smoking-drinking status (15/18, >1110; 20 years >1110; 20 cigarette per day) and long-term professional contact with carcinogen. Mutagen sensitivity assays: The blood lymphocytes were cultivated by standard method. Cultures were incubated for 3 days and then exposed to bleomycin (30 mg/ml) for 5 hours. Cells were harvested by cytogenetic techniques (50–100 metaphase) and mean number of bleomycin induced of chromatid breaks (b/c) estimated. Mutagen sensitivity was determined as the level b/c > 1110; 1. The tumor-distant mucosa and tumors were classified according to the UICC criteria. The sections were immunohistochemically stained using monoclonal antibodies for p53 (clone DO-7, "Daco").

Results: All investigated patients with OSCC were mutagen sensitivity (b/c = 1, one case; b/c > 1, 16 cases) and have a permanent influence on oral mucosa of epidemiological factors of cancer risk. The positive of p53 were all OSCC. In tumor-distant mucosa revealed the progression of histopathological phenotype: from hyperplasia to high dysplasia, carcinoma in situ and OSCC, I degree. p53 was detected in 88.8% cases of tumor-distant mucosa. Negative p53 was found only in cases of hyperplasia. Mild dysplasia showed p53 cells in basal and parabasal layers. In high dysplasia and carcinoma in situ p53 cells prevalent in suprabasal layers of epithelium.

Conclusion: The investigated OSCC were developed in patients with mutagen sensitive phenotype under a permanent influence on oral mucosa of epidemiological factors of cancer risk. Suprabasal p53 expression is strongly associated with grade dysplasia. The mutagen sensitive patients with areas of p53 cells in suprabasal layers of tumor-distant mucosa represent high risk of development of second tumors and require after treatment monitoring and prevention.