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Background: Transmembrane prostate androgen-induced protein 1 (TMEPAI) is a membrane protein that has attracted significant attention of many researchers its involvement in TGF-β signaling pathway which involved in malignant transformation and metastatic tumor progression. We investigated the TMEPAI expression level in gastric adenocarcinomas in comparison to non-tumor mucosa samples and determined its potential prognostic significance.

Materials and methods: Fresh and paraffin-embedded gastric adenocarcinoma samples and paired adjacent normal tissues were collected from gastric cancer patients. Evaluation of the PMEPA 1 gene expression was carried out using RT-PCR. For evaluation of TMEPAI protein expression, monoclonal antibodies (mAbs) were developed by using hybridoma techniques. Specificity of prepared monoclonal antibodies against recombinant TMEPAI and evaluation of its expression in the clinical samples using selected mAbs were performed using immunoblotting and immunohistochemistry.

Results: We have identified more than two-fold increase in gene expression of PMEPA1 in tumor tissue in 44% of patients. The monoclonal antibodies have shown the capacity to specifically recognize the recombinant TMEPAI in HEK293T cell lysates. We also evaluate the ability of the selected antibodies to recognize the target protein in fixed cells by immunocytochemistry. The evaluation of TMEPAI in adenocarcinoma samples collected from gastric cancer patients revealed decreased protein expression. We have observed pronounced expression of TMEPAI in normal gastric epithelial cells, while tumor cells from gastric adenomas and adenocarcinomas samples were mostly negative for target protein expression. We found that gastric epithelium cells lose the TMEPAI expression concurrent with severe dysplasia.

Conclusion: Apparently, the TMEPAI may be a potential biomarker of malignant transformation risk of the stomach epithelium.

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Does LMP1 oncogene expression pattern reflect specific pathology or geographic origin of the EPSTEIN–BARR virus?

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The Epstein–Barr virus (EBV) represents an etiological agent for a number of human benign and malignant tumors. One of the EBV encoded proteins, the latent membrane protein 1 (LMP1), is involved in activation of many signaling pathways and transcription factors leading EBV infected cells to immortalization and transformation.

It's well known that almost all worlds' population is infected with EBV. As usually, infection occurs during early childhood without serious consequences for infected people. At the same time a secondary infection by additional EBV strain(s) occurs quite often. During the in vitro cultivation of peripheral blood lymphocyte from persons infected with multiple strains of the virus, only one of them having LMP1 oncogene with highest transforming potential becomes dominant while the others are eliminated.

To figure out whether pattern of LMP1 expressions reflects the origin of EBV strains, six cell lines from patients with tumors, associated and not-associated with the virus and healthy individuals were established. The nucleotide and deductive amino acid (a.a.) sequences of LMP1 isolates tested were analyzed and compared with those of LMP1 isolates obtained from eight cell lines of African and Japanese EBV-associated Burkitt's lymphomas (BL) origin.

As the result, in four out of six cell lines of Russian origin (2 from patients with lymphoid pathology and 2 from PBLs of blood donors) the low divergent LMP1 B95.8/A variant characterized by a low transforming activity and a small number of a.a. substitutions was detected. For other two cell lines originated from EBV-associated patient with nasopharyngeal carcinoma and not virus-associated Hodgkin's lymphoma patient the LMP1Med- and LMP1China1 variants, characterized by a larger set of mutations and high transforming potential, were found. Low divergent LMP1 variants (B95.8 or B95.8/A) were observed for 13 of 15 LMP1 samples from PBLs of Russian blood donors; in 2 donors highly divergent China1 and NC LMP1 variants were also detected. Among eight cell lines of BL origin three lines were the sources of the prototype EBV strain B95.8 (Jijoye, P3HR1, Raji). From other five cell lines (Daudi, Namalva, Ag 876, NC37 and Akata) LMP1 variants Med- and China1, characterized by a significant number of mutations and high transforming capacity were obtained.

Genetic relationship between LMP1 isolates from cell lines of Russian and BL origin were analyzed by the phylogenetic tree. It follows from the constructed tree that cell lines of Russian and BL lymphoma origin formed two separate clusters located at the tree a distance from each other, indicating genetic proximity for respective groups of cell lines. The data obtained complemented with the results of our previous studies suggest that among Russians represented by cancer patients and healthy individuals,

EBV strains with predominantly low transforming capacity of LMP1 are persisting. These findings are likely can explain the non-endemic nature of the EBV-associated pathologies in Russia.On the other hand, one can speculate that in African countries which are endemic for BL highly oncogenic strains of EBV are dominated, the indirect confirmation of what is the detection in cell lines of BL origin LMP1 isolates having high transforming activity. The results of this study let us also to suggest that LMP1 expression pattern in non-endemic region like Russia does not reflect the type of malignancy but rather reflect their geographic origin.

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Targeting tumor cells with Hsp70 chaperone

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The Hsp70 chaperone is one of the major components of tumor microenvironment displaying multiple not yet established functions. It was shown to stimulate innate and adaptive anti-tumor immunity in a variety of cancer models. These effects were due to active release of endogenous Hsp70 to an extracellular matrix and therefore the extracellular chaperone can be a trigger of multiple events in the whole tumor. Factors inducing Hsp70 release are heat stress, inhibitors of important signaling proteins, anticancer drugs and X-ray treatment. Recently we have shown that delivery of Hsp70 can be efficient pusher of its intracellular analogue to extracellular milieu. In this study we show that intracellular cycling of exo- and endogenous Hsp70s increases the sensitivity of cancer cells to cytotoxic lymphocytes. Moreover, the results of in vivo studies employing B16 mouse melanoma and C6 rat glioblastoma as targets proved the therapeutic relevance of exogenous Hsp70 in intra-tumoral application.

To uncover the mechanisms of the anticancer effect we used inhibitors and markers of intra- and extracellular protein transport. The data of these studies showed multiplicity of pathways using which exo-Hsp70 reaches cytosol and more usable ones was endocytosis. To be exported intracellular Hsp70 employs vesicular structures as well as intra-membrane lipid structures.

Analyzing Hsp70 molecule we found the domain with potential vector activity, i.g. cell penetrating function. This peptide was synthesized and shown to cross a cellular membrane with efficacy exceeding that the whole Hsp70 molecule. Furthermore, the new peptide was used as a carrier for the delivery inside living cells antibody to Hsp70. The resulting construct was found to reduce the resistance of tumor cells to pro-apoptotic effect of staurosporin.

Taking together these data, we can suggest that Hsp70 and its fragments can be effective players in communication between cells assembling functionally active tumor.

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Long non-coding RNA HOTAIR affects DNA methylation patterns in gastrointestinal stromal tumours (GIST)

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Aberrant alterations of DNA methylation patterns have been recognized as early and common events in oncogenesis emphasizing high therapeutic and preventive potential. Concurrent epigenetic silencing of multiple tumour suppressor genes has been reported in different tumours suggesting the existence of a directed program, whereby groups of genes are regulated by promoter methylation. The molecular bases of such a program remain largely unclear. Certain long non-coding RNAs (lncRNAs) have been recently identified that are responsible for target specificity of the histone modification complexes. It remains incompletely understood, however, if lncRNAs may play a role in patterning DNA methylation. In this study, we asked by using gastrointestinal stromal tumours as a model if an epigenetic related *HOX Antisense Intergenic RNA*, or *HOTAIR*, is involved in establishment of DNA methylation patterns. To this end, we first showed high up-regulation of *HOTAIR* in patient samples of high risk compared to low and intermediate risk groups ($n = 66$, $p = 6.7 \times 10^{-6}$), what is supported by the earlier reports on common carcinomas. Highest levels of *HOTAIR* endogenous expression were next detected also in cell lines GIST T1, GIST48b and GIST882. Stable knockdown of *HOTAIR* was achieved in GIST T1 and GIST48b cells by RNAi using lentiviral transduction. As expected epigenetic alterations due to *HOTAIR* knockdown could develop with a delay, genome-wide DNA methylation profiling was performed at passage 12 after the transduction on the Infinium HumanMethylation450 BeadChip platform in triplicates. DNA methylation data were analysed by an R package RnBeads. A total of 218 CpG sites got hypomethylated upon *HOTAIR* knockdown in GIST T1 and GIST48b cells ($\Delta\beta > 0.3$, FDR < 0.05). These included potential tumour suppressors, transcription factors, tumour-specific antigens, genes related to angiogenesis or involved in metabolism. As confirmed by using bisulfite pyrosequencing for a representative locus, DNA methylation of 64% degree at promoter associated CpG sites of the potential tumour suppressor *RASSF1* was almost entirely erased upon *HOTAIR* knockdown in GIST T1 cell line with concomitant up-regulation detected by qRT-PCR. Concordant with earlier reports, *HOTAIR* knockdown led to reduced migration potential in GIST T1 cells. Taken together, the results suggest that *HOTAIR* is one of the factors involved in establishment of specific DNA methylation patterns in GIST. While the molecular mechanism remains to be determined, it is plausible to assume a recruitment of DNA methyltransferases by the Polycomb repressive complex 2, which target specificity is determined by *HOTAIR*. The results further suggest the feasibility of manipulating DNA methylation patterns in a targeted manner and are of potential interest in context of developing epigenetic cancer therapy.