(gamma = −0.636, p = 0.04); the presence of LF with AGC – with the Grade (gamma = −0.530, p = 0.03), histological type (gamma = 0.726, p = 0.02) and the degree of dysplasia GM (gamma = 0.833, p = 0.001). Multiple LF, focal LI and LF with AGC bit more often observed at grade G3-4 and signet ring cell carcinoma (SRCC) (LF 0%, 25%, 42.5% and 55.6%, p = 0.23, LI in 50%, 42.9%, 100%, 66.7%, p = 0.07 and LF with AGC 12.5%, 0%, 50%, 28.6%, p = 0.17, respectively, when G1, G2, G3-4 and SRCC) and diffuse type gastric cancer (LF 20% and 50%, p = 0.12 in 60% and 81.2%, p = 0.23 and LF with AGC 10% and 41.2%, p = 0.07, respectively, for the intestinal and diffuse type gastric cancer). Focal LI and LF with AGC were more common in moderate and severe dysplasia of the gastric epithelium (LI 58.3% and 84.6%, p = 0.14 and LF with AGC 50%, 8.3%, p = 0.02).

A decrease in the three-year relapse-free survival in multiple LF from 75% to 40%, p = 0.04 and in the presence of focal LI – 83.3% to 57.9%, p = 0.17 were observed. In the presence of focal LI, a decrease in the three-year overall survival from 100% to 73.7% was also observed (p = 0.14).

The findings suggest that B-cells may be associated with the factors of GC progression. It is important that the high density of the diffuse B-lymphocytes was typical for early GC, whereas the presence of LI and multiple LF in GM adjacent to tumor was more often observed in diffuse type of GC, poorly differentiated tumors and signet ring cell carcinoma. We believed, that further studies are needed for the understanding of the mechanisms where by the B-cell LI and LF may be related with the histology type and Grade of GC.

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P11

Proteoglycans expression correlates with the phenotype of malignant and non-malignant EBV-positive B-cell lines

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In vitro Epstein-Barr virus (EBV) infects B-cells resulting in immortalization. In vivo the virus resides latent in resting B-cells. Rarely the EBV-host cell interaction may contribute to development of malignant lymphoma. It is well known that both c-myc translocation and the viral infection are observed in patients with EBV+ Burkitt’s lymphoma (BL). Proteoglycans (PGs) are complex glycosylated proteins. They are key components of ECM and play a critical role in cell–cell and cell–matrix interactions. Disruptions of such interactions will affect B cell interaction with surrounding stroma and may thus perturb the cell phenotypes. The purpose of this study was to investigate expression of proteoglycans in EBV+ cell lines with different origins and phenotypes.

We analysed the expression of 12 of the main proteoglycans in primary B cells, lymphoblastoid cell lines (LCLs) generated by EBV infection of normal human B cells in vitro and EBV-positive BL cell lines. An EBV-negative BL cell line was used for reference.

According to RT-PCR analysis, primary B-lymphocytes expressed different PGs, mainly serglycin, CD44, perlecan and syndecan-1. The high expression of PGs in normal B cells probably reflects interactions of these cells with the neighbouring cells and microenvironment. B cell lines which carry EBV, in general, showed lower levels of PGs. The PGs expression pattern was similar in LCLs and in primary B cells, however, distinguished by high levels of perlecan and serglycin and low expression of CD44 in LCLs. BL cells showed the most significant down-regulation of PGs compared to primary B cells. There was a correlation between the type of EBV latency program, and PGs expression. Serglycin was expressed at a low levels in BL-cells with EBV latency III-program compared to LCLs, while in EBV latency I and BL cells both serglycin and perlecan were down-regulated.

Cells with latency I-program show general hypermethylation of the cellular genome in contrast to cells with latency III-program. Thus we explored the possibility of epigenetic regulation of the PG-coding genes by treating cells with 5’-deoxycytidine (5-AzaC, a demethylating agent) and Trichostatin A (TSA, a chromatin structure modulator). There was no significant change in PGs expression upon this treatment in LCLs or in EBV latency III BL cells, while EBV latency I BL cells showed up-regulation of several PGs. This suggests that PGs expression is at least partly regulated by epigenetic mechanisms. Interestingly EBV latency is also partly regulated at the epigenetic level.

Similar trends were observed for the key ECM components (collagen 1A1, fibronectin and elastin). Normal B lymphocytes expressed collagen, fibronectin and elastin, whereas LCLs and BL cells showed no expression of these. Treatment of these cells with 5-AzaC or TSA resulted in similar changes in PGs expression patterns. Up-regulation of ECM components was detected only in EBV latency I BL cells.

Taken together, our data show that proteoglycans are expressed in primary B lymphocytes whereas they are not or only partly expressed in EBV-carrying cell lines, depending on their latency program. Expression of PGs in latency I BL cells is silenced due to hypermethylation, but by another mechanism in latency III BL cells. These results show that PGs expression patterns follow the EBV latency programs. It will be highly interesting to further investigate if EBV and its transformation associated genes are directly involved in control of PGs, as well as how PGs may contribute to major phenotypic properties of EBV-carrying cell lines, such as adhesion, migration and growth in soft agarose – a property associated with the malignant phenotype of BLs.

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P120

Microarray research of allelic imbalance in breast cancers during neoadjuvant chemotherapy
Allelic imbalanced in breast tumor during NCT is typical of many genes in different malignancies. Allelic imbalanced may result allelic deletions (loss of one copy of the locus) or amplification of one allele, resulting in only a single allelic variant of SNP is determined in the tumor in the PCR. Phenomenon of AI in tumors of breast cancer (BC) is considered in one or more genes. The phenomenon of AI during chemotherapy (CT) and especially in the longer format of the gene had not previously been studied.

Thus, the aim of this work was to study microarray imbalanced allele in mammary tumors during neoadjuvant chemotherapy (NCT).

Materials and methods: The study included 26 breast cancer patients with stage IIA – III C. The patients in the neoadjuvant mode received 2–4 courses of chemotherapy regimens FAC or CAX. DNA from 26 paired samples before treatment and operational samples were isolated by dialing QiAampDNA miniKit (Qiagen, Germany). Microarray analysis was performed on DNA chips of high density company Affymetrix (USA) CytoScanTM HD Array, which contains more than 750 thousand SNP. Microarray analysis was performed on SNP genotypes DNA tumor tissue before and after treatment for each patient and recorded as change occurring allelic imbalanced tumor tissue genotype (AA > AB > AB AA BB > AB > AB BB) during therapy.

Results: The frequency of the AI in breast tumor during NCT was highly variable (within 0.9–66.5%) of the studied SNP (6850 – 497,979 SNP). For each patient, frequency shift genotype (homozygous in heterozygous genotype, and vice versa) was calculated as a percentage of all the shifts. Changes in the wild or mutant heterozygous genotypes (AA or BB > AB) were combined into one group, the second group was the sum of the change in the heterozygous genotype homozygous wild genotype or the mutant (AB > AA or BB). We have found that the direction of the AI was significantly associated effect of NCT. In the group of patients with partial regression, the direction of AI change from heterozygous to homozygous genotype during NCT is associated with no response to chemotherapy due to metastasis and occurring of new mutant clones in the tumor.

Conclusion: Allelic imbalanced in breast tumor during NCT phenomenon is massive and may affect up to 67% of SNP. AI may occur in the direction of change from homozygous to heterozygous genotype, and it is associated with a good response to treatment and 100% metastasis-free survival.

Dendritic cells (DCs) are the most potent antigen-presenting cells, which play crucial role in initiation and maintenance of immune response. However, DCs of high-grade glioma patients are characterized by some functional impairment including reduced TNFα-mediated antitumor cytotoxic activity and low migratory activity. Tumor-produced soluble molecules can profoundly affect the differentiation and maturation of DCs and influence the functional activity of DCs. The aim of the present study was to determine the effect of supernatants of primary glioma cell lines on the migration and the cytotoxic activity of IFN-alpha-induced dendritic cells.

Glioma-derived soluble factors pose strong chemoattractants and partially change the cytotoxic activity of IFN-alpha-induced dendritic cells

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Background: For single nucleotide polymorphism – SNP (SNP – Single Nucleotide Polymorphism) is characterized by phenomenon of allelic imbalance (AI). The phenomenon of allelic imbalanced (AI) is typical of many genes in different malignancies. Allelic imbalanced may result allelic deletions (loss of one copy of the locus) or amplification of one allele, resulting in only a single allelic variant of SNP is determined in the tumor in the PCR. Phenomenon of AI in tumors of breast cancer (BC) is considered in one or more genes. The phenomenon of AI during chemotherapy (CT) and especially in the longer format of the gene had not previously been studied.

Thus, the aim of this work was to study microarray imbalanced allele in mammary tumors during neoadjuvant chemotherapy (NCT).

Materials and methods: The study included 26 breast cancer patients with stage IIA – III C. The patients in the neoadjuvant mode received 2–4 courses of chemotherapy regimens FAC or CAX. DNA from 26 paired samples before treatment and operational samples were isolated by dialing QiAampDNA miniKit (Qiagen, Germany). Microarray analysis was performed on DNA chips of high density company Affymetrix (USA) CytoScanTM HD Array, which contains more than 750 thousand SNP. Microarray analysis was performed on SNP genotypes DNA tumor tissue before and after treatment for each patient and recorded as change occurring allelic imbalanced tumor tissue genotype (AA > AB > AB AA BB > AB > AB BB) during therapy.

Results: The frequency of the AI in breast tumor during NCT was highly variable (within 0.9–66.5%) of the studied SNP (6850 – 497,979 SNP). For each patient, frequency shift genotype (homozygous in heterozygous genotype, and vice versa) was calculated as a percentage of all the shifts. Changes in the wild or mutant heterozygous genotypes (AA or BB > AB) were combined into one group, the second group was the sum of the change in the heterozygous genotype homozygous wild genotype or the mutant (AB > AA or BB). We have found that the direction of the AI was significantly associated effect of NCT. In the group of patients with partial regression, the direction of AI change from heterozygous to homozygous genotype during NCT is associated with no response to chemotherapy due to metastasis and occurring of new mutant clones in the tumor.

Conclusion: Allelic imbalanced in breast tumor during NCT phenomenon is massive and may affect up to 67% of SNP. AI may occur in the direction of change from homozygous to heterozygous genotype, and it is associated with a good response to treatment and 100% metastasis-free survival. Apparently, this can be explained by the fact that the change from homozygous genotypes to heterozygous occur due to partial destruction of tumor cells by chemotherapy, resulting in the increase of stromal elements. In contrast, AI in the direction change from heterozygous to homozygous genotype during NCT is associated with no response to chemotherapy due to metastasis and occurring of new mutant clones in the tumor.

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PS2

Glioma-derived soluble factors pose strong chemoattractants and partially change the cytotoxic activity of IFN-alpha-induced dendritic cells

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Dendritic cells (DCs) are the most potent antigen-presenting cells, which play crucial role in initiation and maintenance of immune response. However, DCs of high-grade glioma patients are characterized by some functional impairment including reduced TNFα-mediated antitumor cytotoxic activity and low migratory activity. Tumor-produced soluble molecules can profoundly affect the differentiation and maturation of DCs and influence the functional activity of DCs. The aim of the present study was to determine the effect of supernatants of primary glioma cell lines on the migration and the cytotoxic activity of healthy donor DCs. The study included 11 healthy donors and 7 patients with histologically verified glioma Grade II (2 patients) and glioma Grade IV (5 patients). DCs were generated from peripheral blood monocytes of healthy donors in the presence of GM-CSF and IFN-alpha (IFN-DCs) followed by the addition of lipopolysaccharide (LPS Escherichia coli). Primary glioma cell cultures from tissue samples of studied patients were obtained by mechanical and enzymatic (0.3% collagenase IA) disaggregation followed by culturing in DMEM/F12 medium containing 10% FCS. 7-day supernatants were collected upon reaching the cellular subconfluence. Migratory capacity of donor IFN-DCs in response to supernatants (v/v 25%) of primary glioma cell cultures was measured in trans-well culture chamber. DCs that migrated to the bottom chamber with medium alone as a control or with supernatants were counted by FACS analysis using BD calibration beads. Cytotoxic activity of donor IFN-DCs against tumor cell line HEP-2 was studied using MTT-assay for 24 h at a ratio of DC: HEP-2 1:1. IFN-DCs were preincubated for 2 h with supernatants of primary glioma cell cultures. Migration index of donor IFN-DCs to lymphoid chemokine CCL19 (100 ng/ml) was an average of 6.5 ± 2.1. Migration capacity of IFN-DCs towards concentration gradient of primary glioma culture supernatants was significantly higher. Supernatants of low-grade glioma cultures as well as of high-grade glioma cultures effectively induced chemotaxis of...