revealed remarkable GR-dependent cooperation between CpdA and Bortezomib in suppressing survival of lymphoma cells in vitro and in vivo. Also surprising findings were substantial cooperation in anti-cancer effect of immunosuppressant Rapamycin and CpdA in vitro, and unexpected "dissociated" effect of Rapamycin on GR signaling realized through down -regulation of REDD1, mTORC1 inhibitor. These data suggested high clinical potential of Rapamycin/GC combination in cancer treatment.

(2) SEGRA list extention

We used two approaches to extend SEGRA list: (1) synthesis of CpdA enantiomers and (2) its chemical derivatives. Chemical analogues of CpdA were designed by appending of bulky substituent into benzene ring, alkylation of carbon atom adjacent to chlorine atom or appending of substituents to nitrogen atom. Evaluation of biological properties of enantiomers revealed higher GR-dependent anti-cancer potential of S-CpdA. Cytotoxic and proapoptotic effects of CpdA analogues were comparable with precursor.

(3) Selection of tumor types acceptable for SEGRA treatment.

CpdA was selected for NCI-60 in Vitro Cell Line Screening Project providing direct support to anticancer drug discovery program. It was shown that CpdA affect viability of some adherent cancer cell lines. We demonstrated that CpdA unlike GCs did not modify microenvironment and disintegrate tight junctions between cells decreasing risk of metastasis in case of solid tumors. It demonstrates reasonability of further investigations.

Overall, our data provide the rationale for novel therapy of cancer based on combination of non-steroidal GR modulators with classic and modern chemotherapeutics. Approaches to obtain more SEGRAs were elaborated.

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Clonal evolution of breast tumor during neoadjuvant chemotherapy and metastasis

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Background: There are numerous evidences suggesting that tumor evolution follows the laws of Darwinian evolution, whereby individual tumor cell clones have private genetic aberrations, including chromosomal abnormalities. The combined effect of genetic instability and differential selective pressures of the microenvironment and chemotherapy can result in the creation of new tumor clones (Navin et al., 2011; Ng et al., 2012). The aim of this study is to show breast tumor clonal evolution during neoadjuvant chemotherapy (NAC) using microarray analysis.

Material and methods: Breast cancer patients ($n = 26$) with stage IIA to IIIC (T1-4N0-3M0), were treated with NAC (FAC or CAX regimens). DNA was extracted from 26 samples of tumor tissue derived before or after NAC using QIAamp DNA mini Kit (Qiagen, Germany). Copy Number Aberrations (CNA, deletions and amplifications, or Loss and Gain, respectively) and number of mutant clones were detected in pre- and post-NAC tumor samples using the high density microarray platform Affymetrix (USA) CytoScanTM HD Array. This study was approved by Tomsk Cancer Research Institute review board.

Results: We have revealed that 19% (5/26) of patients during the NAC showed the decrease in the number of mutant clones and CNA frequency right up to their complete elimination (genetic regression) at one case. In 7 (27%) cases chemotherapy had no any effect on number of mutant clones and the frequency of CNA in tumor. In the tumors of 10 patients the elimination of some mutant clones as well as the formation of new clones with deleted genetic material occurred under the influence of chemotherapy. 6 patients have demonstrated the appearance of new tumor clones with gene amplifications which were associated with the development of metastases in 83% of cases (5/6). All other patients ($n = 21$) who has not acquired the new tumor clones with Gain function mutation after NAC did not manifest distant metastasis in 5-year follow-up (Kaplan–Meier, $p = 0.00001$ Log-rank test).

Conclusion: The first time evidence is presented that the formation of new tumor clones may occur during the NAC. Metastasis of breast cancer is associated with the appearance of new clones with DNA amplifications. Detection of these clones allow getting new prognostic factor in breast cancer.

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Proliferative activity, lymphatic and blood vessel density in different clinical stages of melanoma

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Cutaneous melanoma is one of the most aggressive human neoplasms that can quickly metastasize to regional lymph nodes. Currently, prognosis is determined by measuring tumor thickness but more reliable markers for metastatic spread are urgently needed. It is well known that tumors require a microvasculature development in order to grow and metastasize. Angiogenesis and lymphangiogenesis play an important role not only in the tumor growth, but also in the tumor metastasis. As malignancy is a disorder of cellular growth control, the assessment of cell proliferation rates in tumors has intuitive appeal as a prognostic marker. One such biomarker is Ki-67, a cell cycle dependent protein. Recent reports related to its role as a prognostic factor

have been contradictory. The purpose of this study was to investigate lymphangiogenesis, angiogenesis and tumor tissue proliferative activity depending on the clinical stages of melanoma.

The objects of the study were the samples of tumor tissue obtained during surgical treatment of cutaneous malignant melanoma. To analyze tumor angiogenesis, lymphangiogenesis and proliferation, we performed immunostains of the primary melanomas for the vascular marker CD34, for the lymphatic-specific markers LYVE-1, D2 -40 (Podoplanin) and marker of proliferation – Ki-67. Samples of tumor tissue from 40 patients with IA, IB, IIA, IIB, IIIC melanoma stages were fixed in 10% neutral formalin, processed by standard histological techniques and embedded in paraffin. All steps of the immunohistochemical reaction were performed by using BENCHMARK/XT slide stainer (Ventana).

Determination of blood vessel volume density revealed its growth an average of 2 times in peritumoral areas. Similar data were obtained about the location lymphatic vessels. Greater content peritumoral blood and lymphatic vessels, then intratumoral could be detected in all stages of melanoma. In addition more significantly greater volume density both intratumoral and peritumoral blood vessels, than lymphatic vessels was found. A greater degree expression of endothelium lymphatic vessels markers Podoplanin, compared with the LYVE-1 was shown. It was noted an increasing volume density of the peritumoral blood and lymphatic vessels in primary tumors with expansion clinical stage melanoma. Three levels of proliferative activity of tumor tissue were determined (low, an average degree and high Ki-67 expression). Results of the analysis have shown, that the high proliferative activity corresponded to significant content of blood and lymphatic vessels. High level of these markers was observed in patients with regional lymph nodes metastases.

In recent years there have been appeared publications suggesting that lymphatic vessel density (particularly in a peritumoral location) and lymphatic vessel invasion are predictors of sentinel node metastasis and poorer survival. It was noted, that "larger, carefully conducted and well-reported studies that confirm these preliminary findings are required before it would be appropriate to recommend the routine application of costly and time-consuming immunohistochemistry for lymphatic markers in the routine clinical assessment of primary cutaneous melanomas". In our opinion, one must consider not only the lymphatic vessels density, but also blood vessels, and the tumor tissue proliferative activity.

Conclusion: This study has shown that the complex markers of lymphangiogenesis (Podoplanin), angiogenesis (CD34) and proliferation (Ki-67) may be predictors of high risk early melanoma metastasis.

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The human oncogenome evolution advances ahead of the evolution of human protein-coding genome and other specific gene classes

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Background: Earlier we showed that at least some of nucleotide sequences with tumor-specific expression are evolutionary novel (reviewed in [A.P. Kozlov, 2014]). In this paper we performed the study of the relative evolutionary novelty of human oncogenes, all protein-coding genes, genes encoding tumor antigens, tumor suppressors and tumor-associated genes using homology searches in genomes of different taxa in human lineage.

Materials and methods: The following databases were used as a source of human genes: oncogenes – COSMIC (574 genes), tumor suppressors – TSGene (636 genes), all tumor-associated genes – allOnco (2116 genes) and NCG (2001 genes), cancer-testis (CT) antigen genes – CTDatabase (276 genes) and all annotated human protein coding genes – Genome assembly GRCh38 (21694 genes). The list of cancer vaccine antigen genes was retrieved from paper of Cheever et al., 2007, where 75 cancer antigens were ranked according to their potential suitability for anticancer vaccines. Some of cancer antigens are non-protein molecules, mutant or fusion-proteins. Thus, we examined the evolutionary novelty of only 58 protein-coding cancer vaccine antigen genes. To analyze the evolutionary novelty of the explored genes the HomoloGene release 68 tool and ProteinHistorian tool were used. The HomoloGene tool searches the orthologs in 11 taxa of the human lineage (Eukaryota, Opisthokonta, Bilateria, Euteleostomi, Tetrapoda, Amniota, Boreoeutheria, Euarchontoglires Catarrhini, Homininae, H.sapiens) and the ProteinHistorian tool searches the orthologs in 16 taxa of the human ligeage (Cellular Organisms, Eukaryota, Opisthokonta, Bilateria, Deuterostomia, Chordata, Euteleostomi, Tetrapoda, Amniota, Mammalia, Theria, Eutheria, Euarchontoglires, Catarrhini, Homininae, H.sapiens). To analyze the statistical significance of data Fisher's exact test was used.

Results: Several curves of taxonomic distribution of orthologs of different classes of human genes have been generated. A set of curves forms a peculiar picture where different curves are organized in a definite order. The curves never intersect after Bilateria. The uppermost position occupies the curve which describes the oncogene orthologs distribution. Right below the oncogene curve, the distribution curve of tumor suppressor genes orthologs taxonomic is located, and the difference between these curves is significant. The distribution curves of other tumor-associated genes orthologs overlap with tumor suppressor curve. The medium position in the whole picture is occupied by the distribution curve of orthologs of all human protein-coding genes. Below this curve the distribution curves of orthologs of different tumor antigens are located. The first below the medium curve is tumor vaccine antigen curve, then CT and CT-X antigen genes orthologs distribution curves are located. Thus at any given timepoint the relative proportion of oncogene orthologs described by distribution curve is higher than of any other studied class of human genes.

Conclusion: 1. The evolution of human oncogenome advances ahead of all other human gene classes. 2. On the other hand, the evolution of tumor antigen gene classes goes behind the rest of human gene classes, i.e. tumor antigens genome is more evolutionary novel.

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