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Hyperspectral system imaging for detection of cancer cells

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In recent years, hyperspectral system imaging (HSI) has been used as a tool for the detection of nanomaterials in biological matrices. Here we present using the HSI for obtaining of hyperspectral images in a complex and highly dispersive environment, such as cell cultures. This information is necessary to develop an effective strategy for the detection of cancer cells. HSI records the scattering of light in the visible and near-infrared (VNIR, 400–1000 nm) regions in each pixel of the image field. Using the HSI it is possible to find out the spectral profile that is unique to the each studied object. We studied human colon carcinoma (HCT-116) and human prostate adenocarcinoma (PC3) cell lines. Thus, we collected a scattering intensity database that provided the spectra of cancer cells. The spectral profile for HCT-116 showed a bimodal peak at 550 nm and 675 nm. For PC3, the spectrum showed a band with a maximum at 600 nm and with a noticeable shoulder at 675 nm. The spectral profile for different cancer cells is significantly different, that allows for optical and spectral differentiation. The work was done at the expense of subsidies allocated as part of the state support of KFU in order to increase its competitiveness among the world's leading scientific and educational centers, and through funding under the state 16.2822.2017/4.6 and MD-6655.2018.4. Also, the work was partially carried out with the financial support of the Russian Foundation for Basic Research and the government of the RT grant № 18-44-160001.

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Induction of cell death in human melanoma cell lines by the combination of p14ARF plus interferon-β gene transfer

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In our cancer immunotherapy approach, we aim to induce both cell death and an anti-tumour immune response upon gene transfer. Previously, we have shown that combined p19Arf (functional partner of p53) and interferon-β (IFNβ, pleiotropic cytokine) gene transfer resulted in elevated levels of cell killing associated with markers necroptosis, immunogenic cell death (ICD) and immune activation in a mouse model of melanoma. Here we present a critical advance in our understanding of how this approach impacts human melanoma cells. We have constructed non-replicating Ad5 vectors with constitutive expression of either human p14ARF or IFNβ and applied these viruses to the UACC62 and SK-Mel-29 cell lines. For both, elevated levels of killing were encountered upon combined gene transfer as compared to single gene treatment as measured by the accumulation of hypodiploid cells, activation of caspases 3/7 and annexin-V staining. Strikingly, pan-caspase inhibition using Z-VAD-FMK peptide blocked cell death in UACC62, but not SK-Mel-29, indicating a non-apoptotic mechanism of cell death in the latter case. In situ gene therapy of s.c. UACC62 tumours in nude mice revealed superior inhibition of progression when four doses of combined gene therapy were applied, an effect that could be extended with additional virus injections. While combined p14ARF+IFNβ gene transfer is beneficial for cell killing and control of tumour progression, much remains to be studied with regard to the mechanism of cell death and immune activation in response to our gene therapy approach in human cells. Supported by the Sao Paulo Research Foundation (FAPESP), CNPQ and CAPES.

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Antitumor properties of artificial microvesicles from mesenchymal stem cells overexpressing TNF-related apoptosis inducing ligand

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Almost all human cells release extracellular vesicles (EVs), spherical micro- and nanoparticles, which are separated from the cell surface and participate in intercellular communication. EVs are a promising tool for the delivery of bioactive molecules for therapeutic purposes. One of the promising cytokines with anti-cancer properties is TNF-related apoptosis-inducing ligand (TRAIL) which is able to selectively induce apoptosis in malignant but not normal cells. The ideal cell type for the production of EVs is human mesenchymal stem cells (MSCs) as they exhibit a homing behaviour to tumor niches and MSC-isolated EVs retain the ability of the parental cells to migrate toward tumor sites. Genetic modification of MSCs with TRAIL gene and the subsequent production of EVs from them can be a promising approach for cancer treatment. This study was performed in the accordance with approved ethical standards and current legislation (protocol approved by the Committee on Biomedical Ethics of KFU (No.3, 03/23/2017)) and supported by grant MK-236.2019.4. In this study, MSCs with TRAIL overexpression were treated with cytochalasin B to increase the yield of EVs. Cytochalasin B-induced artificial microvesicles (CIMVs-TRAIL) were positive for CD44, CD90 and CD105 MSC surface markers, but CD29 and CD73 expression was significantly decreased (about 10%). TRAIL expression in CIMVs was confirmed by qPCR and Western blot. Using TEM and flow cytometry CIMVs-TRAIL were shown to be mostly 50–200 nm in diameter which is comparable with natural EVs. CIMVs-TRAIL exhibited significant antitumor activity in SH-SY5Y tumor cells culture in vitro.

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Cancer-derived exosomes loaded with ultrathin palladium nanosheets for targeted bioorthogonal catalysis

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The transformational impact of bioorthogonal chemistries has inspired new strategies for the in vivo synthesis of bioactive agents through non-natural means. Among these, Palladium (Pd) catalysts

have played a prominent role in the growing subfield of bioorthogonal catalysis by producing xenobiotics and uncaging biomolecules in living systems, and new exciting Pdcatalyzed reactions and applications continue to emerge. However, delivering catalysts selectively to specific cell types still lags behind catalyst development. Towards this goal, we have developed a bio-artificial device consisting of cancer-derived exosomes loaded with Pd catalysts by a novel method that enables the controlled assembly of Pd nanosheets directly inside the vesicles. This new hybrid system mediates Pd-triggered dealkylation reactions in vitro and inside cells and displays preferential tropism for their progenitor cells. The use of Trojan exosomes to deliver abiotic catalysts into designated cancer cells creates the opportunity for a new targeted therapy modality: exosome-directed catalyst prodrug therapy, whose first steps are presented herein with the cell-specific release of the recently approved anticancer drug panobinostat.

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WEE1 and CHK1 gene silencing using Polypurine Reverse Hoogsteen Hairpins

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We investigated the inhibition of CHK1 and WEE1 genes, using Polypurine Reverse Hoogsteen hairpins (PPRHs). PPRHs are gene silencing molecules, composed by two mirror repeat polypurine domains, linked by a pentathymidine loop and bound by intramolecular reverse-Hoogsteen bonds. Different PPRHs were designed, 3 directed to WEE1 and 4 to CHK1. All PPRHs were tested individually at 100 nM in HeLa cells in cell viability assays. Five PPRHs reduced cell viability around 80% and were tested in PC3 cell line with similar results. We observed a dose-dependent effect in reducing cell viability when using HpWEE1Pr-T and HpCHK1I1-C. In addition, time-course assays, showed that HpWEE1Pr-T and HpCHK1I1-C were cytotoxic with only 6 hours of cell incubation at 100 nM. After 20 hours of incubation with HpCHK1I1-C and HpWEE1Pr-T, there was an increase in apoptotic cell population of 3-fold. The treatment of HpWEE1Pr and HpCHK1I1-C reduced mRNA levels of their target genes between 1.6-2-fold. Moreover, we observed a decrease of 50% WEE1 protein levels after 24 h incubation of HpWEE1Pr-T and a decrease of 50% of CHK1 protein levels after 15 h of incubation of HpCHK1I1-C. In addition, we observed the formation of two splice variants of CHK1 mRNA after 24 h of incubation with HpCHKI1I1-C. In conclusion, Inhibition of WEE1 and CHK1 genes using PPRHs results in decreases in mRNA and protein levels and increases in apoptosis. Moreover, HpCHK1I1-C promotes the appearance of new splicing variants. Thus, PPRHs can be used to inhibit genes involved in replicative stress as anti-cancer gene therapy.

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Production of mouse mesenchymal stem cell lines with Luciferase and Katushka2s reporter gene expression for bioluminescence imaging

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Mesenchymal stem cells (MSCs) are a major component of the tumor microenvironment and play a key role in promoting tumor progression. MSCs have been shown to exhibit a homing behavior toward tumor sites that makes it perspective vehicles for anti-cancer agent delivery, particularly chemotherapy drugs. The ffLuc-encoding gene cloned from the Photinus pyralis is the most studied and well characterized bioluminescent reporter gene. In this study mouse MSCs (mMSCs) that express the luciferase reporter gene (ffLuc) or Katushka2S were produced. mMSCs were isolated by enzymatic digestion with collagenase. This study was performed in the accordance with approved ethical standards and current legislation (the protocol was approved by the Committee on Biomedical Ethics of KFU (No. 3, 03/23/2017)) and with support by RFBR grant 18-34-00738. The cells were largely positive for MSC surface markers (CD44, CD90, CD29, CD105, CD73 and Sca-1) and negative for hematopoietic stem cell markers. Recombinant lentiviruses LV-ffLuc and LV-Katushka2S were produced by co-transfection of the HEK293T packing cell line. The viral titer was determined by flow cytometry of cells transfected with lentiviruses carrying Katushka2S. mMSCs were transduced with recombinant lentiviral vectors encoding ffLuc or Katushka2S gene. Resulting mMSCs-ffLuc cell line was selected with puromycin for 10 days. The relative intensity and stability of the firefly luciferase signal in mMSCs-ffLuc were analyzed by using ONE-Glo™ Luciferase Assay System. Obtained mMSCs-ffLuc cell line with a stable luminescent signal will be further primed with various chemotherapeutic drugs and its antitumor properties will be analyzed in vitro.

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Replicating retroviral vectors spread on the human retinoblastoma SNUOT-Rb1 cells and induce cell death

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A couple of viruses have been developed as oncolytic virotherapy to kill infected tumor cells directly. However, a couple of problems of oncolytic virotherapy such as early viral clearance by host immune system and frequent attenuation of the viral infectivity raised the need of new tools for virotherapy. As a promising tool for cancer treatment, replicating retroviral vector (RRV) is known to have high selectivity into tumors and stability in gene transfer because RRV can replicate only in dividing cells and has extremely low immunogenicity. Retroviral-mediated transfer of the herpes simplex virus type 1 thymidine kinase (HSV1-TK) gene or yeast cytosine deaminase (yCD) gene into glioma followed by treatment with prodrug has been widely used for glioma gene therapy clinical trials. One of the most common childhood cancer, Retinoblastoma (Rb), is an intraocular tumor that grows rapidly and poses a threat to sight and life. In this study, we examined the potential of a split-RRV system encoding HSV1-TK and yCD gene for retinoblastoma gene therapy. We, here, show the feasibility of RRV for retinoblastoma gene therapy.

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Development of lipid nanoparticles for the mRNA-mediated cancer immunotherapy