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In vitro probing of human refractory prostate cancer cells by microRaman spectroscopy

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Vibrational spectroscopy is a reliable analytical tool for the characterization of biological systems, as it provides accurate information at the molecular level. In particular, Raman confocal microspectroscopy of cell cultures allows a non-invasive probing of living cells [1] with a high sub-cellular spatial resolution, that enables observing and mapping the distinct cell components without the use of dyes or probes [2].

The aim of this preliminary work is to apply microRaman spectroscopy for probing living prostate cancer cells (PC-3), while preserving cell integrity and function.

MicroRaman spectra were recorded in a Renishaw InVia Raman microscope spectrometer, coupled to a RenCam CCD detector, using a Leica 100x lens. An exciting laser power of ca. 10 mW at the sample was applied, for 10 accumulations and 60 s of exposure time. Spectra of living PC-3 cells in exponential growth (3×10^4 cells/cm^2) were obtained in quartz windows, focusing on the cytoplasm, the membrane and the nuclei.

This study allowed to get access to the chemical composition of the tested *in vitro* cells. Further assays are ongoing, aiming at assessing the biodistribution and effect of Pt- and Pd-based anticancer agents, such as cisplatin [cis-$Pt(Cl_2(NH_3)_2]$ and Pd_2-Spm [Spm=$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$], as a function of dosage and incubation time [3]. This will hopefully lead to a better understanding of the drug-cell interactions inducing cell-growth inhibition and/or death by apoptosis.

References

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SW04.S16–115
Candidate breast cancer DNA vaccine: Design of polyepitope antigen and evaluation of it's expression in human dendritic cells

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Breast cancer is one of the most common malignancies in women and is the second leading cause of cancer death. In the modern era of breast cancer treatment, immunotherapy has emerged as an effective tool for improving clinical outcomes. We propose to induce breast-cancer-specific CD8+ T cell response after transfection of dendritic cells with synthetic polyepitope DNA vaccine. Polyepitope antigen (PA) encoding DNA sequence has been designed from HLA-A*0201 restricted CTL–epitopes previously described and predicted with TEpredict software in the structure of two tumor-specific breast cancer antigens – HER2 and Mammoglobin 1. PolyCTLDesigner software was used for optimization of target antigen structure including proteolytic sites, localization signals and Gag-epitope for monitoring expression and metabolic stability of the target immunogen. DNAs encoding PA and native antigens HER2 and Mammoglobin 1 were synthesized and cloned into pcDNA 3.1 vector.

LPS-free plasmid DNAs were purified using previously described protocol (RP №2408729). The absence of LPS in plasmid preparation was demonstrated by LAL-test. Dendritic cells (DCs) were obtained from human peripheral blood by density gradient centrifugation, plastic adherence with subsequent GM-CSF/IL-4 stimulation. DCs were transfected with plasmids using MATra-Reagent and maturated using the appropriate cytokine cocktail. Mature transfected DC's were characterized by microscopy and flow cytometry using antibodies against CD80, CD86, CD83, CD11c, CD14, HLA-DR, CD83. Efficacy of DC transfection was evaluated using real time PCR, level of target antigen expression was estimated using antibodies against Gag-epitope and antibodies to parent proteins. The data obtained demonstrate that DCs loaded with the PA are able to activate breast-cancer-specific CD8+ T cells, but additional study is required to confirm cytotoxicity of the DNA-vaccine against breast cancer cells overexpressed HER2 and Mammoglobin 1.

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CYP2D6 genotype and tamoxifen response in pre and postmenopausal Thai women with hormone responsive breast cancer

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Tamoxifen is effectively used as adjuvant therapy for hormone responsive breast cancer patients. Cytochrome P450-2D6 (CYP2D6) enzyme involves in metabolizing tamoxifen to active metabolites. CYP2D6 polymorphisms have been suggested to affect tamoxifen efficacy among Europeans and some Asians. In this study, we preliminary investigated the associations of CYP2D6 metabolizing enzymes genotypes with tamoxifen response measure as breast cancer-free interval time (refer to as recurrence). DNA extracted from patient blood was used for genotyping seven CYP2D6 single -nucleotide polymorphism using microarray-based pharmacogenetic testing. Genotype combinations were used to categorized CYP2D6 metabolism phenotypes as poor, intermediate, and extensive metabolizers (PM, IM, and EM, respectively, n = 48). Associations of CYP2D6 metabolism phenotypes with breast cancer-free interval were assessed using Kaplan-Meier analysis. Cox proportional hazards models were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs). No association between CYP2D6 metabolism phenotypes and breast cancer-free interval was observed among all patients. However, the shorter disease free survival (DFS) was significantly shorter in post menopausal patients with PM phenotypes (homozygous variant TT when compared to those with heterozygous CT or homozygous CC(EM) at nucleotide 100C>T and 1039C>T(CYP2D6*10)(Log-rank test p = 0.046). The association of increase the risk of recurrence, however was not significantly difference among PM and IM