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TUE-078
eEF1A1 and eEF1A2 hetrodimerization by confocal microscopy and fret analysis

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The eukaryotic translation elongation factor 1A (eEF1A) catalyzes the first step of the protein synthesis elongation cycle [1]. Two isoforms of the protein (eEF1A1 and eEF1A2), sharing 92% amino acid homology, are known expressed in a tissue specific manner: eEF1A1 is widely expressed in almost all tissues whereas eEF1A2 is normally expressed only in non-proliferative tissues (brain, heart, skeletal, muscle). Beyond their canonical role in protein synthesis, both isoforms have been reported to be involved in other non-translational functions. eEF1A1 isoform seems to play a pro-apoptotic role whereas eEF1A2 appears to protect from apoptosis suggesting its potential function as putative oncogene [2]. Previous studies identified eEF1A Raf-mediated phosphorylation sites (S21 and T88) during survival response mediated by epidermal growth factor receptor (EGFR). However phosphorylation of S21 occurs only in presence of both eEF1A isoforms, suggesting that the eEF1A isoforms can heterodimerize. A docking model supported the formation of a possible heterodimer between eEF1A1 and eEF1A2. In particular, this model showed that the M-domain of one isoform was in contact to the G-domain of the other and vice versa. The heterodimer formation somehow could induce a conformational change in one or in both eEF1A isoforms that allows the phosphorylation of S21 [3]. The heterodimerization hypothesis was investigated by carrying out sensitized emission FRET experiment that involves the non-radiative transfer of energy from an excited state donor fluorophore to a nearby acceptor and the energy transfer efficiency ($FRET_{eff}$) was evaluated using FRET and colocalization analyzer ImageJ plug-in software [4]. HeK293 cells were transfected with pcDNA3.1 construct containing the human eEF1A2 cDNA ingineered with a C-terminal His_6-tag. FRET measurements via confocal microscopy, was carried out using mouse anti-His antibody and rabbit anti-human EF1A-1 antibody. Confocal analysis showed colocalization of the eEF1A isoforms within the cytoplasm with a stronger merge signal at the level of plasma membrane. FRET analysis indicated a specific interaction between endogenous eEF1A-1 and transfected eEF1A1-2 at the level of the plasma membrane. These results strongly confirmed that the eEF1A isoforms might interact by possibly forming a dimer that undergo to phosphorylation regulated by C-Raf [3].

References

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Keywords: Elongation Factor 1A, FRET, Protein - protein interactions.

TUE-079
Effect of down-regulation of STEAP1 by siRNA in cell cycle and apoptosis of human LNCaP prostate cells

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Introduction: The six transmembrane epithelial antigen of the prostate 1 (STEAP1) is over-expressed in several types of tumors, particularly in prostate cancer. STEAP1 can be found in the plasma membrane of epithelial cells, at cell–cell junctions, but also in the cytoplasm with less intensity. Although its physiological functions are yet to be ascertained, STEAP1 appears to take part on intra- and intercellular communication. Several studies have pointed out that STEAP1 has a great potential as an immunotherapeutic target, highlighting its role on prostate pathophysiology. It is well known that sex steroid hormones, namely dihydrotestosterone (DHT), are not only involved in maintenance of prostate physiology but also promote proliferation of prostate cancer cells. In addition, it was recently demonstrated that DHT is involved in regulation of STEAP1 gene. This way, we hypothesized that the effect of DHT on cell proliferation and apoptosis may be dependent on STEAP1 levels in cancer cells.
Materials and Methods: LNCaP prostate cancer cell line was cultured under an atmosphere of 5% CO_2 and 37°C. To access the effects of DHT and STEAP1 on cell proliferation and apoptosis, cells were transfected with STEAP1 siRNA and either treated with 0 or 10 nM DHT for 48 h. Control group included non-transfected LNCaP cells and treated with 0 nM DHT. Cell cycle and apoptosis analysis was performed by flow cytometry, using propidium iodide and FITC annexin V staining, respectively. Cell proliferation was also investigated through MTS assay.
Results and Discussion: STEAP1 gene silencing with a specific siRNA appears to induce cell cycle arrest and decrease proliferation when compared to control group. Hormonal treatment with DHT induces proliferation in the non-silenced cells, as it was previously described by others. When comparing siRNA transfected cells treated with DHT with non-transfected cells, it appears that DHT impairs cell proliferation, diminishing the number of cells on S-phase. Apoptosis analysis is underway.
Conclusions: STEAP1 may influence prostate cell proliferation, and the effect of DHT on prostate cells seems to be dependent of STEAP1 levels. However, more assays are being carried out in order to clarify the role of STEAP1 in cell proliferation and apoptosis.
Keywords: prostate cancer, STEAP1.

TUE-080
Effect of doxorubicin loaded CS MNPs on cell proliferation in sensitive and doxorubicin resistant MCF-7 cell lines

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Targeted drug delivery is a promising alternative in cancer therapy to increase the efficacy of drugs and reduce the destructive side effects of classical chemotherapy. In order to obtain an effective targeted delivery system for Doxorubicin, chitosan coated magnetic nanoparticles (CS MNPs) were synthesized (Unsoy *et al,* 2012). CS MNPs maintain pH dependent drug delivery while providing targeting of drugs to the tumor site under magnetic

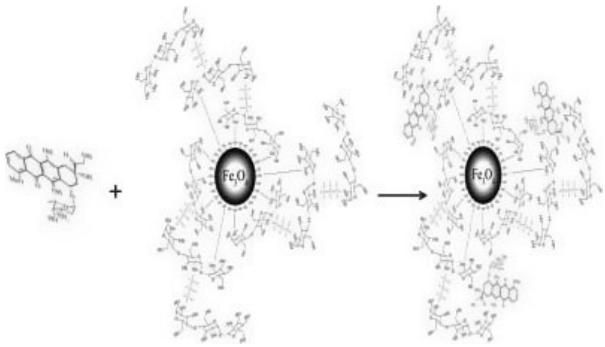

Fig. 1. Schematic representation of Doxorubicin (a), CS MNP (b) and of Doxorubicin loaded CS MNP (c).

field. The anti-cancer agent Doxorubicin, which is an antracycline antibiotic, was loaded onto CS MNPs (Figure 1). As maintained from drug loading, release, and stability characterization studies, CS MNPs have pH responsive release characteristics and are quite stable at physiological pH.

Anti-proliferative effects of Doxorubicin loaded CS MNPs were investigated by XTT cell proliferation assay on MCF-7 and MCF-7/Dox cells at increasing concentrations. IC_{50} values of free Doxorubicin and Doxorubicin loaded CS MNPs indicated that drug loaded nanoparticles not only increase the efficacy of drug but also overcome Doxorubicin resistance in MCF-7/Dox cells. Consequently, CS MNPs can be effectively used for the pH dependent release of Doxorubicin in cancer cells. Results of this study can pave the way for the development of pH responsive targeted drug delivery systems to overcome the limitations of conventional chemotherapy.

Keywords: Doxorubicin, drug resistance, breast cancer, cytotoxicity.

TUE-081
Effect of holothurians triterpene glycosides, cucumariosideA2-2 and frondoside A, on human prostate cancer

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Prostate cancer is the most common malignancy among men and the second leading cause of cancer related deaths. In the beginning the metastasized stage can be controlled by hormone deprivation. However, the vast majority of tumors become castration-resistant in the course of treatment. Although new drugs e.g. Abiraterone, Cabazitaxel and Enzalutamid have been successfully applied in this situation, cancer cells again acquire resistance to these substances with patients facing a dismissal prognosis. Thus, the search for new natural or synthetic compounds with selective anticancer properties remains a high scientific priority.

Holothurians triterpene glycosides cucumarioside A_2-2 and frondoside A were isolated from the holothurian *Cucumaria japonica* and *Cucumaria frondosa* and kindly provided by Drs. Avilov and Silchenko (PIBOC FEB RAS, Vladivostok, Russian Federation). Anticancer properties and mechanism of action were evaluated in the castration-resistant human prostate cancer cell line PC3: Trypane blue staining, MTT test and colony formation assay were applied for cytotoxicity and cell proliferation studies. Influences on cell cycle and apoptosis were evaluated by flow cytometry and western blotting analysis. The effect of triterpene glycosides on protein expression was analysed by 2D-gel electrophoresis followed by MALDI-MS.

EC_{50} for cucumarioside A_2-2 and frondoside A was 3 μM and 2,5 μM determined by trypane blue method and 3,3 and 3,0 μM by MTT assay, respectively. Cucumarioside A_2-2 and frondoside A caused cell cycle arrest in G2/M phase. A relative increase of the cells in the Sub-G_0 phase of the cell cycle was observed indicating cell death. Indeed, both glycosides induced apoptosis in concentrations equalling EC_{50} or less reflected by caspase-3 and caspase-9 activation. Inhibition of tumor cell colony formation of up to 40% was observed after glycoside treatment when compared to controls. In addition, incubation of PC3 cells with triterepene glycosides resulted in a distinct regulation of protein expression: Both substances up-regulated Keratin, type II cuticular Hb1 and Interleukin-1 beta, and down-regulated expression of Cathepsin B and Heterogeneous nuclear ribonucleoprotein A/B, which are involved in metastatic formation, tumor cell invasion and malignant growth.

In conclusion, cucumarioside A_2-2 and frondoside A are promising novel substances showing high efficacy in castration-resistant prostate cancer.

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Keywords: Anticancer activity, Cell cycle, Triterpene glycosides.

TUE-082
Effect of phytoestrogen emodin on doxorubicin-induced cytotoxicity and apoptosis on tumorigenic MCF-7 and non-tumorigenic MCF-10A human breast cells

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Emodin (3-Methyl-1,6,8-trihydroxyanthraquinone), is a phytoestrogenic component of *Rheum and Polygonum* plant extracts which has been used to treat several diseases since ancient times. It has been shown to have anti-microbial, anti-oxidant and anti-cancer effects in nature. The anti-tumor drug doxorubicin, a widely used chemotherapeutic agent, is used for the treatment of many cancer types including lung, gastric, ovarian and breast cancer. In this study, the effects of pre-, co- and alone treatment of doxorubicin and emodin in MCF-7 and MCF-10A cell lines were investigated.

MCF-7 and MCF-10A cells were cultured in the presence of various concentrations of emodin and doxorubicin at 6, 24 and 72 hours. The effect of emodin varies according to the presence of doxorubicin (pre-treatment, co-treatment, post-treatment) on both cell lines. Emodin pre-treatment (0.4 and 4 μM) for 24-hour prior to doxorubicin treatment (0.1, 0.83, 2.5 μM) caused to increase in cell viability of MCF-10A cells, comparing to doxorubicin alone treatment. Whereas no effect was observed in MCF-7 cells. Emodin post- and co-treatment with doxorubicin for 72-