NanoBiotechnology and Cancer Research

Paracrine interaction drives EMT and lethal progression of prostate cancer: from biology to therapy

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Prostate cancer progression toward the development of lethal bone and soft tissue metastases are major cause of morbidity and mortality in patients. Based on the characterization of a series of isogenic human prostate cancer cell lines recapitulating the lethal progression of human prostate cancer metastases, we found prostate cancer cells with bone metastatic potential, expressed bone-like proteins (osteomimicry), including osteocalcin, osteopontin, bone sialoprotein, osteonectin, receptor activator of NF-κB (RANK), RANK ligand (RANKL), and/or osteoprotegerin (OPG).

β2-Microglobulin (β2-M), a light chain of MHC class 1 molecule, was identified as a host factor from tumor microenvironment, driving Epithelial to Mesenchymal Transition (EMT), osteomimicry, lethal bone and soft tissue metastases. A novel β2-M receptor, the hereditary hemochromatosis gene HFE (a MHC-like protein), is responsible for the regulation of intracellular iron through binding to transferrin and transferrin receptor, and regulating the status of oxidative stress of cancer cells. Anti-β2-M monoclonal antibody (anti-β2-M Ab) was found to target effectively EMT and cancer bone colonization in mice.

RANKL, another target gene of β2-M, was shown to participate in osteoclastogenesis associated with cancer bone metastases. RANKL expressed by metastatic castration resistant prostate cancer (CRPC) cells and tissues participated directly in prostate cancer bone metastases. These data are consistent with the clinical results where targeting RANKL-RANK axis with bisphosphonates (Zoledronic Acid) or Denosumab (a RANKL monoclonal antibody) reduced bone pain and skeletal related events in CRPC patients treated with androgen deprivation therapy.

New approaches targeting osteomimicry and the converging signaling between RANKL/RANK and HGF/SF/c-MET will be described from the perspective of developing novel therapies for the control of bone and soft tissue metastasis and the lethal progression of prostate cancer in patients (supported by NCI PO-1 and RO-1 grants and PCF Challenge Award.

Keywords: prostate cancer, control of bone and soft tissue metastasis

NanoBioTech Studies at ICB (UA): RECOOP collaborative potentials

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Aim: The following applied research approaches are used at ICB: 1) development of novel efficient bio-targeting carriers for drug and gene (siRNA) delivery; 2) development of novel carriers for delivery of water-insoluble drugs; 3) application of novel labeling materials for visualization of drug delivery, action, and clearance in the organism; 4) development of nano(micro)particles for cell isolation and separation.
Methods: Novel polymeric nanocomposites (1) and superparamagnetic nanoparticles (2) were synthesized at Lviv National Polytechnic University (1), Kyiv National University (1), and Institute of Macromolecular Chemistry (IMC, Prague, Czech Republic) (2). Those materials were functionalized for providing them bio-targeting characteristics. Flow cytometry, light and fluorescent microscopy, Western-blot analysis of expression of proapoptotic proteins, *in vivo* toxicity studies in mice, and chemotherapy treatment of tumor-bearing mice were applied at characterization of the developed drug carriers. Specific approaches for detection, selection and addressed targeting the apoptotic cells have been also developed.
Results: To address the NanoBioTech research tasks within the RECOOP Association, superparamagnetic nanoparticles were synthesized at IMC, functionalized at ICB, and used to monitor phagocytic activity of macrophages. Besides, similar particles were bio-functionalized by specific lectins, and used to isolate different murine lymphoma cells. Polymeric nanoparticles were designed and decorated with specific lectins for addressed targeting the apoptotic cells. Effective gene delivery to microorganisms and mammalian cells was achieved by the developed polymeric nanocomposites. They showed low toxicity and no mutagenicity.
Conclusions: ICB research team is ready to discuss common design of novel nanocomposites, their bio-functionalization, and application for achieving different joint research tasks within collaborative biomedical projects of the RECOOP Association.
Keywords: nanocarriers, drug delivery, gene delivery, treatment, diagnostics, apoptosis.

Designing, synthesis, structural and optical properties of ultrasmall inorganic markers doped with lanthanide ions for bio-medical applications

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Aim: Introducing to medicine and biology concept of optical markers in tremendous way has changed the recent status of these two important disciplines. This was mainly due to strong development in imaging techniques which recently allow us to investigate both static as well dynamic properties of living cells, their components and their interactions with external factors.
Method and results: Recently used molecular markers including organic dyes, fluorescent proteins or chelates containing lanthanide ions have several significant limitations. One of the alternatives for molecular markers are inorganic quantum dots (ie. CdSe, CdS) which are commonly used in many academic works. However, even if they are much better from physico-chemical point of view, from the application point of view at this moment they are rather useless mainly because of their high risk of toxicity. One of the solution combining advantages of both concepts is to make nontoxic inorganic nanocrystals doped by lanthanide ions.
Conclusion: In this work, we will present optical results obtained for $NaYF_4$, $NaGdF_4$ and GdOF nanocrystals doped by different lanthanide ions (Eu, Tb, Nd). The aim of this work was to design and to synthesize these markers and to understand physical processes responsible for their emission/excitation and to optimize these processes to the physical limits.
Keywords: nanocrystal, rare earth ions, GdOF

Nanoparticles for monitoring differentiated stem cells

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Aim: It is important in regenerative medicine to noninvasively track stem cells to evaluate their therapeutic effect and grafting location to rule out side effects. Magnetic nanoparticles are therefore being developed as labels visualized by MRI in *in vivo* cell tracking. Before evaluating the clinical potential of nanoparticles in cell transplantation, the effect of these particles on cultured cells should be assessed. As a model, we chose human mesenchymal stem cells (hMSCs) as they are widely used in the regeneration of connective tissues (bone, cartilage and fat). We studied the basic properties of hMSCs labeled with superparamagnetic nanoparticles: labeling efficiency, cell growth, proliferation, migration and differentiation.
Methods: hMSCs were labeled with neat or poly (L-lysine) (PLL)-coated superparamagnetic γ-Fe_2O_3 nanoparticles prepared by an original procedure. Labeling efficiency, cell proliferation and migration were determined by counting the number of Prussian Blue-stained and unstained cells using a cell analyzer. Cell migration was studied using SDF1 factor as a chemoatractant. The osteogenic, chondrogenic and adipogenic differentiation potential of nanoparticle-labeled MSCs was also determined.
Results: The efficiency of cell labeling for PLL-coated and uncoated γ-Fe_2O_3 was 91.5 % and 45 %, respectively. The magnetic label slightly reduced cell proliferation during the first 10 h; however, the final PLL-γ-Fe_2O_3-labeled cell index was high after 24 h. Compared to unlabeled cells, cell migration towards the chemoattractant was not affected. No difference was found in the gene expression of LPL and PPARG adipogenic markers, while osteogenic gene expression (ALPL and RunX2) slightly decreased. However, histological examination revealed that osteogenic (Alizarin Red), chondrogenic (Alcian Blue) and adipogenic (Red Oil O) differentiation were not affected.
Conclusions: Even a highly efficient cell label, PLL-γ-Fe_2O_3, does not significantly affect basic stem cell properties and therefore can be considered as a suitable contrast agent for *in vivo* monitoring of transplanted stem cells in different applications, such as the transplantation of MSCs into cartilage or bone defects.
Keywords: magnetic, stem cells, labeling

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Immobilization of recombinant human arginase I onto gold and silver nanoparticles and their potential use

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Aim: Metal nanoparticles (NPs), such as gold (Au) and silver (Ag), have recognized importance in chemistry, physics, and biology due to their unique optical, electrical, and photothermal properties. AuNPs have photothermal properties that can be exploited for localized heating which results in drug release, thus increasing their potential for therapeutic applications of some malignant diseases (melanoma, hepatocarcinoma).
The aim of the present study was to immobilize the recombinant human arginase I on AuNPs and AgNPs and to characterize the obtained enzyme-NPs: analyses of their size, structure, enzymatic activity and stability.
Methods: The size and structure of the AgNPs and AuNPs were characterized using TEM, AFM and XRD analyses.
Results: The synthesis of silver and gold nanoparticles was carried out by reduction of silver nitrate by glucose and reduction of tetrachloroauric acid by sodium citrate, respectively. Recombinant arginase I was immobilized using the carbodiimide method on the surface of NPs functionalized with ω-mercaptohexadecanoic acid. Recombinant human arginase I was successfully linked on both NPs with a binding efficiency of 85% for AgNPs and 87% for AuNPs (in the range of added enzyme concentration 0.15-0.5 mg/mL). The nanocomposite-bound arginase was used to construct potentiometric and amperometric biosensors for monitoring of arginine level during arginase-based enzymotherapy of some cancers.
Conclusions: Thus, the synthesized gold and silver nano-carriers have a stabilizing effect on recombinant human arginase I due to its fixation on the surface of the NPs, preventing enzyme inactivation. So, the immobilization procedures open extensive possibilities for the construction of very sensitive and stable biosensors for wide biochemical applications. A high thermostability of immobilized on NPs arginase I preparations and a wide working pH range of this enzyme is predicted to be a perspective tool for bioanalytical purposes, namely, for arginine monitoring in pharmaceuticals, in food products and in blood.
Keywords: silver and gold nanoparticles, covalent enzyme immobilization.

Multispectral quantum dot labeling detects elevated C-MET cell signaling mediators in castration resistant and invasive human prostate cancers

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Aim: To test the hypothesis that activation of c-MET-mediated signaling in prostate cancer cells supports cancer cell growth and survival and promotes progression and metastasis. We focused on the simultaneous detection and quantification of 5 proteins in the c-MET activation pathway, receptor activator of NF-κB ligand (RANKL), VEGF, NRPLN-1, p-c-MET and p-p65 (NF-κB) and epithelial to mesenchymal transition (EMT) known to be associated with human prostate cancer progression and bone metastasis.

Methods: We established a novel technology of multiplexed quantum dot labeling, MQDL, to detect the expression and activation of multiple c-MET pathway and EMT mediators in tissue specimens and subject to signal intensity quantification. Three experimental systems were used: 1) human prostate cancer cell model exhibiting activated c-Met signaling and bone metastasis; 2) xenograft tissues from an established LTL313 castration-resistant human prostate cancer (CRPC) model; and 3) clinical prostate cancer tissue specimens. Nuance software was used for image capturing, unmixing and inForm software for per cell base intensity quantification of the five prostate cancer progression associated proteins in the c-MET activation pathway, RANKL, VEGF, NRPLN-1, p-c-MET and p-p65 (NF-κB) and EMT biomarkers EpCAM, N-cadherin and RANKL.

Results: Cell based MQDL quantification data showed that the c-MET activation mediators, RANKL, VEGF, NRPLN-1, p-c-MET and p-p65 (NF-κB) and mesenchymal biomarkers, N-cadherin and RANKL were all elevated in CRPC human prostate mouse xenograft model and in the invasive human prostate cancer with statistical significance. Results were confirmed by real-time RT-PCR and western blots in a metastatic human prostate cancer cell model.

Conclusions: Our results showed that activation of c-MET-mediated signaling occurs in prostate cancer cells through increased phosphorylated c-MET in all three tested systems. The downstream survival signaling network was mediated by NF-κB and EMT driven by RANKL, in clinical prostate cancer specimens and the xenograft model. MQDL is a powerful tool for assessing biomarker expression and it offers molecular insights into cancer progression at both the cell and tissue level with high degree of sensitivity.

Keywords: multiplexed quantum dot labeling, c-MET mediators, castration-resistant prostate cancer, epithelial to mesencymal transition, metastasis

The role of epigenetic regulation in cervical carcinogenesis

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High-risk or oncogenic human papillomaviruses (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, etc) are causally linked to the development of cervical cancer. The E6 and E7 oncoproteins of high-risk HPVs are responsible for the transforming activity of the virus. The virus genome is usually found in an episomal form in benign and premalignant lesions, while it is frequently integrated in the host cell genome in malignant cancers. The E6 and E7 oncogenes are always retained and expressed in the transformed cells.
During cervical carcinogenesis, genetic and epigenetic alterations disrupting the cell cycle control are needed to acquire immortal phenotype and to progress further to overt malignant and invasive phenotype. The stepwise accumulation of these alterations is manifested in the well-defined clinical stages.
Epigenetic alterations include those heritable (covalent) modifications of DNA and histone proteins that do not result in changes of the genomic DNA sequence. In vertebrates, CpG methylation of DNA acts in context with post-translationally modified histones and other chromatin-remodelling factors to establish the characteristic gene expression profile of individual somatic tissues (2). In cancer, the aberrant functions of chromatin modifier enzymes result in altered DNA methylation patterns and histone modifications and the epigenetic alterations involved in uncontrolled cellular proliferation and viral expression during the multistep process of cervical carcinogenesis.