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Posters

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Characterisation of clonal and subclonal allelic imbalance at the HLA locus in a 31 patient multi-region profiled primary/metastasis clear cell renal cell carcinoma cohort

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Introduction: Metastasis is the primary cause of death in cancer. Large-scale studies of metastatic disease have not included analysis of matched primary tumours, which are required to distinguish between tumour clones with and without metastatic potential. Although the treatment of primary tumours has become more successful, five-year survival rates for metastatic renal cancer remain low at 8%. An improved understanding of the genetic differences between primary and metastatic tumours could reveal distinct therapeutic vulnerabilities between local and metastatic disease, which if exploited could improve treatment and/or prevent metastasis. Immune evasion is required for tumors progression and metastasis.

Aims: We investigated whether genomic alterations causing the loss of Human Leukocyte Antigen (HLA) alleles would facilitate immune evasion and subsequently promote proliferation and metastasis.

Methods and results: We acquired the ability to decipher potential modes of metastatic progression through simultaneous analysis of 418 primary and 278 metastatic biopsies from 31 renal cell carcinoma patients. Multiple regions from each tumour were biopsied giving us clonal resolution. AI was investigated using fluorescently labelled STR oligonucleotides that were polymorphic within the HLA locus. We showed AIHLA was significantly selected for within the metastases. Immunohistochemical analysis on 897 tumour biopsies stained for the proliferation marker Ki67 showed AIHLA did not associate with increased tumour proliferation rates. The detection of genomic alterations in tumour biopsies is confounded by infiltration from stromal DNA. Therefore, we developed a novel bioinformatics tool that purified and Characterised Allelic Imbalance in Tumours (CAIT). This was achieved through mathematical deduction of signals originating from stromal DNA. CAIT increased AIHLA detection, providing insights on AIHLA's prevalence and timing.

Discussion: These results suggest AIHLA could promote metastatic progression. The characterisation of AIHLA could increase our understanding of drug-resistance mechanisms and inform the development of immunotherapeutic agents targeting neoantigens.

Biography

Faiz Jabbar studies at University College London as a fifth-year medical student. He has completed a BSc in Immunology, Infection and Cellular Pathology in 2018, where he investigated the ability of renal cancer to evade the immune system and how it correlated with metastatic disease, cancer evolution and heterogeneity.

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Cell-cell and cell-substratum contacts: Impact on MAPK signaling molecules involved in the regulation of cancer and stem cell functioning

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Background: Anoikis ("homeless") is an apoptotic form of programmed cell death induced by detachment from the extracellular matrix. Resistance to anoikis is emerging as a hallmark of cancer. During anticancer therapy, cell-cell and cell-substratum contacts play important roles in the cell fate determination. Increased resistance to anoikis enables malignant cells to survive during migration into secondary tissue and metastasize. Normal cells, however, do not possess such resistance. This is why molecular mechanisms of anoikis regulation may serve as a target both in reducing metastatic cancer growth and in increasing stem cell therapy efficacy. Superfamily of Mitogen-Activated Protein Kinases (MAPKs) regulates cell functions such as proliferation, differentiation and programmed cell death. The regulatory events initiated by extracellular contacts also involve MAP kinase cascades. In the present study, the impact of extracellular contacts on activation of signaling molecules AKT, MAPKs (ERK, JNK, p38) as well as transcription factor cJun in different mammalian cell *in vitro* model systems – lung adenocarcinoma A549, primary lung cancer cell and muscle-derived stem cell lines – was investigated.

Methods: Human non-small cell lung cancer adenocarcinoma A549 cells were obtained from Cell Lines Service (Germany). Human primary lung cancer cell lines were established from surgical material (regional bioethical approval no. 158200-18/5-1024-537). Myo stem cell line was derived from adult rabbit thigh muscle anterior tibia in Institute of Biochemistry (Lithuania). Cells were cultivated at 37ºC and 5% CO2 in IMDM medium supplemented with 10% FBS and antibiotics.Inhibition of cell adhesion. Trypsinized cells were suspended in CO2-independent medium and incubated in a shaker for 24 hours. Then cells in suspension were fractionated in order to obtain non-aggregated and aggregated cell fractions and lysed for protein analysis by Western blot method. Mode of cell death was determined by fluorescence microscopy using a mixture of acridine orange/ethidium bromide fluorescent dyes to identify the apoptotic (anoikis) cells. The role of specific signaling pathways during anoikis was evaluated using specific inhibitors: LY294002 (PI3K/Akt pathway), AZD6244 (ERK pathway), SP600125 (JNK pathway), PF573228 (FAK inhibitor) and MG132 – proteasomal inhibitor.

Findings: The results indicate that cancer cells are more resistant to anoikis when compared to stem cells. Cells in aggregates survived better than single cells in suspension. Survival of both kinds of cells was dependent on protein kinases AKT and ERK. Anoikis diminished AKT phosphorylation in both kinds of cells, whereas phosphorylation of p38 and JNK increased. However, loss of cell-substrate contacts differently affected ERK activation in tested cancer and normal stem cells. Cell-substrate-independent ERK activation was observed in oncogenic RAS harboring A549 cells.

In addition, we have demonstrated that expression of transcription factor c-Jun was dependent on cell culture density in a way that cell-cell contacts promoted proteasomal degradation of c-Jun. Furthermore, phosphorylation of prosurvival kinases AKT and ERK increased during myogenic differentiation of Myo cells making them more resistant to anoikis.

Conclusion & Significance: The results suggest that targeting of prosurvival kinases AKT and ERK during anoikis should be different in different cancer and stem cells. Manipulation of MAPK activity could be engaged in the improving efficacy of cancer therapy.

Acknowledgement: This work has received funding from the European Regional Development Fund (Project No. 01.2.2-LMT-K-718-01-0072) under grant agreement with the Research Council of Lithuania (LMTLT).

Biography

Audrone Kalvelyte is currently a senior research associate in the Institute of Biochemistry, Vilnius University, Lithuania.

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Assessment of anticancer drug impact on survival signaling pathways in primary lung cancer cell lines

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Background: Patient-derived cancer cell cultures/lines is a promising approach in predicting clinical response. As demonstrated recently in a large-scale analysis, patient derived primary cancer cell lines harbor the same genetic alterations that are found in patient's tumor *in vivo*. Signaling molecules of Mitogen-Activated Protein Kinases (MAPKs) and PI3K/AKT/mTORC pathways, key regulators of cell proliferation as well as differentiation and death, are activated by various and different oncogenic mutations and are common for many cancers. Deregulated signaling pathways are associated with resistance to chemotherapy. Therefore, combination of conventional chemotherapeutic drugs with inhibitors of intracellular signaling molecules is a promising strategy for cancer treatment. In this study MAPK ERK and AKT kinase-targeted drugs and their combinations with conventional chemotherapeutics were tested on lung adenocarcinoma cell line A549 and a panel of lung cancer primary cell lines from different patients.

Methods: ERK and AKT-targeted drugs (ERK inhibitor SCH772984, trametinib and selumetinib (MEK), capivasertib (AKT), idelalisib (PI3K)) and their combinations with conventional lung cancer chemotherapeutics (cisplatin, paclitaxel and docetaxel) were tested on A549 cell line and a panel of different primary cell lines derived from surgical specimens of lung cancer patients (Regional bioethical approval no. 158200-18/5-1024-537). Cell functional parameters such as viability, proliferation, apoptosis, morphology was assayed by microscopy, MTT, resazurin reduction and crystal violet staining methods. Cancer stem cell associated and EMT markers, signal transducing molecules expression and phosphorylation in response to drug treatment were evaluated by Western blotting and immunocytochemistry.

Results: *In vitro* drug testing in a series of dose-response experiments showed differences in drug sensitivity between cell lines. Constitutively active/phosphorylated ERK1/2 (pERK1/2) and AKT (pAKT) were further stimulated by chemotherapeutics in studied cancer cells, but the level was cell, drug and concentration-dependent. ERK and AKT-targeted drugs resulted in cell proliferation inhibition but had a minor effect on conventional chemotherapeutic drug-induced cell death when applied singly, whereas combined ERK and AKT inhibition led to increase in anti-proliferative and pro-apoptotic responses, though again, depending on the cell type. The efficacy of targeted drugs was confirmed by reduced target activity/phosphorylation in tested cells. Moreover, the results obtained demonstrated the existence of compensatory feedback loops between two signaling pathways in those cells: inhibition of PI3K/AKT pathway resulted in activation of MAP kinase ERK and, vice versa, inhibition of MEK/ERK pathway augmented pAKT level.Dependency on HER2, oncogenic RAS, amplified c-Met, integrin-β1, MLCK, myosin-IIA, ribosomal protein S6, PUMA, mevalonate pathway has been reported in different cellular systems as being involved in defining this phenomenon. In present study, we showed that the effect might be dependent on the signals induced by extracellular contacts and the strength of chemotherapeutic stimulus.

Significance: Our findings provide more insight into molecular mechanisms of interactions between pro-survival pathways and may suggest additional ways in diminishing cancer cell resistance.

Acknowledgement: This work has received funding from the European Regional Development Fund (Project No. 01.2.2-LMT-K-718-01-0072) under grant agreement with the Research Council of Lithuania (LMTLT).

Biography

Aurimas Stulpinas is currently a junior researcher in the Vilnius University, Lithuania.

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Allicin promotes antimycobacterial activity of macrophages during Mycobacterium tuberculosis infection

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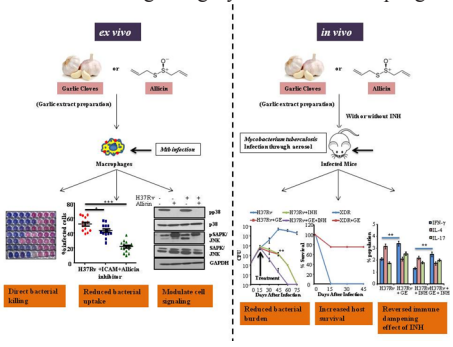
The emergence of drug-resistant Mycobacterium tuberculosis (M.tb) strains has severely hampered global efforts towards tuberculosis (TB) eradication. The internationally accepted therapy "Directly Observed Treatment Short-course (DOTS)" is lengthy, and incorporates risks for the generation of drug-resistant M.tb variants. Multiple and extremely Drug-Resistant (MDR and XDR) variants of TB are now widespread throughout the globe, and Totally Drug Resistant (TDR) strains have appeared. Therefore, new classes of antibiotics are urgently needed to combat these deadly organisms. Historically, garlic is known to kill mycobacterial strains, and its active compound, allicin, kills various microorganisms. Here we have shown that allicin not only reduced the bacterial burden in the lungs of mice infected with Mycobacterium tuberculosis (M.tb), but also induces strong anti-tubercular immunity.

In the present study, the anti-mycobacterial and immunomodulatory activity of garlic extract and its pure constituent allicin were demonstrated based on several *in vitro* and *in vivo* experiments in murine model of tuberculosis. Furthermore, the validation of study was done by immunoblots showing the modulation of MAPK and SAPK/JNK signaling by allicin in macrophages. Here, we report that allicin/garlic extract exhibits strong anti-mycobacterial responses *in vitro* and *in vivo* against drug-sensitive, MDR and XDR strains of TB. In addition to direct killing, allicin also induced pro-inflammatory cytokines in macrophages. Moreover, allicin/garlic extract treatment in murine models of infection resulted in induction of strong protective Th1 response, leading to drastic reduction in mycobacterial burden. These results indicated that allicin/garlic extract has both antibacterial and immunomodulatory activity. Furthermore, garlic extract reversed the immune dampening effects of frontline anti-TB drugs. Allicin/garlic extract alone or as an adjunct to classical antibiotics holds great promise for treatment of drug-sensitive as well as drug-resistant TB. These results warrant further study and validation of allicin for treatment of TB.



Biography

Mona Singh is currently pursuing her Ph.D. from Jawaharlal Nehru University, Delhi, India. She was the alumni of Banaras Hindu University and University of Delhi, while pursuing her Bachelor and Masters degrees, respectively. Her research focus lies in studying the immunomodulatory effect of different compounds using mice models. She has published her papers in several reputed journals.

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Increased serum visfatin levels in benign and malignant thyroid diseases

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Statement of the problem: Thyroid gland is affected by a wide variety of diseases ranging from hyperplastic to neoplastic, autoimmune, or inflammatory. Thyroid dysfunction is accompanied by changes in the thyroid hormone levels, which in turn affect the secretion of adipokines. Nicotinamide phosphorybosiltransferase (NAMPT), also known as visfatin, is an adipokine overexpressed in many chronic inflammatory auto-immune diseases. Our study aims to assess the expression of visfatin in patients with thyroid diseases and its relationship with disease-related characteristics.

Methodology: Study participants were forty patients with papillary thyroid cancer, twenty patients with nodular goiter and twenty healthy controls. Serum levels of visfatin were quantitatively determined upon diagnosis by ELISA. Differences between patient groups and associations with demographic and disease characteristics were statistically evaluated.

Findings: Detectable serum visfatin levels were observed in 18/40 (45%) patients with thyroid cancer, 4/20 (20%) patients with benign thyroid disease and 7/20 (35%) healthy controls. Median visfatin levels were significantly higher in patients with malignant or benign thyroid disease compared to controls ($p<0.05$). No significant differences in visfatin levels were observed between patients with thyroid cancer and those with benign disease, as well as between patients with thyroid cancer and controls. Visfatin was not correlated to gender, age or BMI of participants. In patients with thyroid disease, visfatin was not correlated to tumor size, thyroid mass, thyroid volume, multicentricity, Bethesda classification and family history of thyroid disease.

Conclusion: The lack of concordance in existing findings, regarding the differences in visfatin concentrations between thyroid patients and healthy individuals, may be attributed to the heterogeneity of the disease. Our preliminary data of significantly higher levels of visfatin in patients with benign and malignant thyroid diseases further supports the implication of visfatin in the heterogeneous pathogenesis of the thyroid gland.

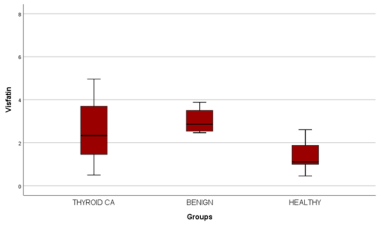

Figure 1. Serum Visfatin levels in patients with malignant and benign disorders and healthy controls

Biography

Bletsa is a specialized Medical Doctor in the field of Laboratory Medicine. In the last ten years she had been working in microbiology,hematology, biochemistry, immunology and blood donation department, carefully analyzing test results for a proper diagnosis. She obtained her PhD diploma on September 2013. Since June 2018 she has been working in the Research and Diagnostic Center of the Hellenic Anticancer Institute. Our main objective is to find new biomarkers with diagnostic, prognostic, predictive and therapeutic value.

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Immunotherapy: Strategies for expanding its role to treat all major tumor sites

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Immunotherapy is widely regarded to have the ability to transform the treatment of cancer, not least because it avoids the many limitations of chemotherapy and radiotherapy. As immunotherapy effectively harnesses the immune system, in principle it should be able to treat a broad range of tumor types independent of the underlying histology or driver mutations. However, to date immunotherapy has only demonstrated efficacy in a select group of cancers and usually in a minority of patients with those cancers, limiting its use as a treatment. This can be partly attributed to additional immunosuppressive mechanisms in the tumor microenvironment that help promote and maintain a state of T cell exhaustion. As such, numerous strategies are being employed to combat these evasive mechanisms and expand the role of immunotherapy to treat all major cancers. In particular, the exploration of combinatory immunotherapies is a promising area of research, and includes the combination of immune checkpoint inhibitors with cytotoxic therapies, cancer vaccines and monoclonal antibodies against other co- inhibitory and co-stimulatory receptors. Strategies to improve the homing, extravasation and survival of CAR-T cells in the tumor microenvironment are also being investigated. Furthermore, the development of immunotherapies targeted to one or multiple neoantigens unique to a specific tumor may act to enhance anti-tumor immunity, as well as reduce immune-related adverse events (irAEs). As immunotherapy evolves to become a mainstay treatment for cancer, it is imperative that optimum treatment regimens that maximize bioavailability and efficacy, whilst limiting toxicity, are developed. Foremost, appropriate biomarkers must be identified, in order to help tailor combinatory immunotherapies to the individual patient and hence pave the way to a new era of personalized medicine.

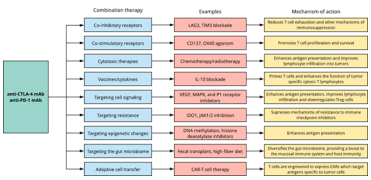

Figure 1: Combinatory immunotherapy approaches and their synergistic mechanisms of action

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IL-10 mediated microvascular and epithelial perturbations in rejecting mouse airway allografts

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Microvascular injuries during inflammation are key cause of transplant malfunctioning and permanent failure, which play a major role in the development of chronic rejection of the transplanted organ. Inflammation induced microvascular loss is a promising area to investigate the decisive roles of regulatory and effector responses. The present study was designed to investigate the impact of IL-10 on immunotolerance, in particular, the microenvironment of the allograft during rejection.

Here, we investigated effects of IL-10 blockade/ reconstitution, and serially monitored regulatory T cells (Tregs), graft microvasculature and airway epithelium in rejecting airway transplants.

We demonstrated that blocking/reconstitution of IL-10 significantly modulate CD4+FOXP3+ Tregs, microvasculature and airway epithelium during rejection. Our findings further highlighted that blockade of IL-10 upregulated proinflammatory cytokines, IL-2, IL-1β, IFN-γ, IL-15 and IL-23, but suppressed IL-5 secretion during rejection, however, reconstitution of IL-10 significantly upregulated CD4+FOXP3+ Tregs, tissue oxygenation/blood flow and airway repair.

Collectively, these findings demonstrate a potential reparative modulation of IL-10 during microvascular and epithelial repair which could provide a vital therapeutic window to rejecting transplants in clinical practice.

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Angiotensin II induces Reactive Oxygen Species (ROS) generation and apoptosis in lymphoma EL4 cells

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Background/aim: Renin angiotensin system (RAS) is implicated in cardiovascular diseases. Ang II infusion induces hypertension and vascular damage especially endothelial dysfunction, inflammation and oxidative stress. Angiotensin II receptors are expressed in immune cells like T cells and monocytes. Tumour become more aggressive and dangerous in parallel to angiogenesis development witch induce intravasation and extravasation cells going from primary tumour to blood and then from blood to colonize new site and induce metastasis. Macrophages play a pivotal role in inflammation and cancer microenvironment. We aim to study the effect of angiotensin II in Macrophage polarization and cancer microenvironment related to innate immunity.

Materials and methods: Malignant lymphoma EL4 cells were incubated for 24h, 48h and 72h with conditioned media of macrophages stimulated with Angiotensin II (1Um) for 24h. We determine the effect of Angiotensin II stimulate macrophages in Reactive Oxygen Species (ROS) generation using CellRox kit, viability and apoptosis using immunofluorescence and cell phenotyping using flow cytometry.

Results and conclusion: There was a significant increase in ROS generation in cells incubated with Angiotensin II compared to control. Angiotensin II increases significantly apoptosis time dependently from 24 to 72h and change cell CD inflammatory markers after 24h incubation. These differences may be caused by significant increase in ROS generation in cells incubated with Angiotensin II compared to control.

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