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(>5 years) distant relapse, indicating that disseminated tumour cells can reside for prolonged periods of time as “dormant” single cells or non-productive micrometastasis in distant sites. Despite late relapse being a common occurrence in breast cancer, the mechanisms underlying the maintenance of the dormant state and the subsequent escape from dormancy remain poorly elucidated. This can be attributed to a lack of animal models, which can recapitulate the natural progression to dormancy.

Material and Methods: In this study we have identified two human breast cancer cell lines which either in a spontaneous metastasis setting or when inoculated directly into the circulation, gives rise to dormant cells in the lungs of immunocompromised mice. In addition to this we have established an in vitro dormancy assay and an ex vivo lung slice approach in order to interrogate the stromal factors identified from the in vitro and in vivo models, that are required for the progression from a dormant state to a proliferative one.

Results and Discussion: In both the in vitro and in vivo models we have demonstrated that these dormant cells can be stimulated to develop productive macrometastasis. Using an ex vivo lung slice approach, we were able to maintain viability of the lung slices for up to 21 days with the breast cancer cells remaining dormant as seen in vivo. This provides a platform to strictly manipulate stromal factors and monitor the emergence from dormancy in live tissue.

Conclusion: Using these models we can interrogate the contribution of the microenvironment to the emergence from metastatic dormancy with an overall aim to identify and develop clinically relevant novel strategies to suppress or prevent the development of metastatic disease.

No conflict of interest.

[495] Anticancer effects of lipid encapsulated Bilberry

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Rapidly accumulating laboratory and clinical research evidence indicates that anthocyanins have anticancer activity and more intriguing, the evaluation of bilberry anthocyanins as chemo-preventive agents is progressing. These applications are collectively due to the anthocyanins up-regulating tumor suppressor genes, inducing apoptosis in cancer cells, repairing and protecting genomic DNA integrity, which is important in reducing age-associated oxidative stress, as well as improving neuronal and cognitive brain function. Bilberry anthocyanins have pronounced health effects, even though they have a low bioavailability. To increase the bioavailability, Bilberry was encapsulated in 8 nm diameter liposomal nanospheres, called NutraNanoSpheres (NNS), at a concentration of 2.5 mg/50 µL. These Bilberry NNS were used to study the apoptotic/cytotoxic effects on K562 cancer cells. Flow cytometric fluorescent quantification of the uptake of Propidium Iodide in a special cell viability formulation into dead K562 cells was used to determine the effects of Bilberry on the viability of K562 cells. The concentrations of Bilberry that showed the highest levels of percentage inhibition, relative to the control populations, were biphasic, showing 60–70% inhibition between 0.018–1.14 mg/ml (n = 6) and 60% inhibition at 80 mg/ml. The lowest % inhibition (30%) occurred at 40 mg/ml. The LD50 was determined to be 0.01–0.04 mg/ml of Bilberry per 10⁵ K562 cells at 72 hr. of cell culture exposure. At 48 hr. incubation, the highest % inhibition was only 27%, suggesting a long-term apoptotic event being involved. These levels, which showed direct cytotoxic effects, were 10 times lower than what is required for the Bilberry that is not encapsulated. The 10 fold increase in bioavailability with the Bilberry NNS and its water solubility show the feasibility of using Bilberry NNS in cancer patient clinical trials.

No conflict of interest.

[496] A novel pre-clinical model for imaging cancer-associated inflammation

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Background: Chronic inflammation is a hallmark of most, if not all cancers and is a major risk factor in the development of many cancer types. Immune cells create a microenvironment that promotes the survival and sustained proliferation of cancer cells as well as encouraging malignant transformation, angiogenesis and resistance to therapy. Consequently, it is vital to develop a pre-clinical model in which we can assess tumour-related inflammation and correlate this with disease progression and therapeutic response. To address this need we are developing the two-stage carcinogenesis model, for use in pre-clinical PET and MR-imaging. In this model, heterogeneous tumours with complex microenvironments develop in situ, in immune-competent animals. Metastatic progression to invasive squamous cell carcinoma (SCC) occurs in 10–15% of tumours, permitting the assessment of malignant transformation

in the context of an intact immune system. Because the model generates multiple tumours, the effects of different topically applied treatments can be evaluated on the same animal, enabling efficient pharmacodynamic biomarker validation. Treatment of these tumours with an established pharmacological inhibitor (XMD8-92) of the fundamental cell-signalling protein ERK5, decreases intra-tumoural inflammation (particularly macrophages) eliciting a substantive decrease in tumour burden.

Materials and Methods: Anti-ERK5 therapy (XMD8-92) was employed as a tool to modify the inflammatory tumour microenvironment. We then evaluated the effect of XMD8-92 on intra-tumoural inflammation using the PET tracers [¹¹C]PK11195 and [¹⁸F]FDG. We also employed DW-MRI (ADC) to evaluate changes in cellularity in order to determine if this technique can detect changes in tumour immune cell infiltrate.

Results: Inhibition of intra-tumoural inflammation, via anti-ERK5 therapy, resulted in a reduction in tumour-associated uptake of [¹¹C]PK11195 and [¹⁸F]FDG. Diffusion-weighted MRI revealed that control tumours show increases in ADC over time, consistent with a change in cellularity that may reflect inflammation. XMD8-92-treated tumours show stabilisation of ADC relative to control.

Conclusions: The TSPO-based PET tracer [¹¹C]PK11195 can track specific changes in intra-tumoural macrophage populations. Anti-ERK5 therapy may also alter tumour glucose consumption. DW-MRI can give rise to biomarkers indicative of inflammation.

No conflict of interest.

[497] The role of lysyl oxidase activity in ccRCC cell adhesion and mobility in vitro

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Background: Lysyl oxidase (LOX), an extracellular matrix remodelling enzyme, has a role in normal embryonic development and connective tissue function. However, LOX promotes tumour progression and metastases although it may also have tumour-inhibitory effects, which depend on the location and the transformation status of the tumour, and the cell type involved. Our previous studies found higher expressions of LOX in clear cell renal cell carcinoma (ccRCC) compared to adjacent normal renal tissues. Clear cell renal cell carcinoma arise from epithelial cells of the proximal convoluted tubules of the nephron and account for approximately 80% of all renal malignancies. Unfortunately, the link between LOX activity and ccRCC cell adhesion and metastasis remains unclear.

Material and Methods: ccRCC cell line, Caki-2 cells were cultured in McCoy's 5A complete growth medium supplemented with 10% FBS in a humidified 5% CO₂ atmosphere at 37 °C. Various concentrations (50 to 200 µM) of beta-aminopropionitrile (BAPN), an irreversible inhibitor of LOX, were used to inhibit LOX activity in ccRCC cells for 24 to 48 hours in vitro. Untreated cells cultured under the same condition were used as control. The activities of LOX were tested using a fluorometric method. BAPN treated and untreated cells adhesion to fibronectin were analyzed. The expression of genes involved in tumour metastatic signalling pathways were assessed by quantitative RT-PCR. Western blotting analyses were performed to evaluate the expressions of matrix metalloproteinases, MMP2 and MMP9, following LOX inhibition in Caki-2 cells.

Results and Discussion: Our results showed that BAPN inhibited LOX activity in ccRCC cells in a dose-dependent manner in vitro. LOX inhibition was associated with a significant increase in ccRCC cell adhesion to fibronectin in vitro. The inhibition of LOX enzyme elevated the expression of metastatic regulatory genes, MTSS1 and NME in ccRCC cells. In contrast, expressions of MMP2 and MMP9 proteins in ccRCC were reduced following LOX inhibition. These results indicate that LOX may promote ccRCC cell progression and metastasis by modulating metastatic gene expressions. Thus, targeting LOX by inhibiting its activity may be a new strategy in preventing ccRCC metastasis.

Conclusion: LOX activity affects ccRCC cell adhesion to extracellular matrix, and is associated with cancer cell metastasis. This study suggests LOX may be a potential therapeutic target in ccRCC patients.

No conflict of interest.

[498] GRP94 protein and prosurvival autophagy, the Achilles heel on brain metastasis progression

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Introduction: GRP94 is a 94-kDa glycoprotein abundant in the endoplasmic reticulum (ER) and is induced by glucose starvation. Overexpression of GRP94