PO-199 LYSYL OXIDASE IN HEAD AND NECK CANCER: METASTASIS AND THERAPY RESPONSE

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Introduction Hypoxia occurs when tumours out-grow the vasculature. It can induce therapy resistance, contribute to metastasis and is a potential biomarker of radioreistance (RR) in Head and Neck Squamous Cell Carcinoma (HNSCC). However, neither identification of patients who might benefit from hypoxia-modification, nor a full understanding of the molecular changes that contribute to metastasis/RR, has been achieved. A recent study by our lab was able to link hypoxic volume in patients with a genetic signature correlating to poor progression-free survival. Lysyl oxidase (LOX) was chosen for further investigation as it has been previously implicated in poor prognosis and hypoxia-induced metastasis. LOX has not however been extensively studied in the context of RR. Additionally, factors other than hypoxia have been shown to contribute to LOX’s regulation, but, their relative contributions to metastasis and RR have not been defined.

Material and methods LOX was found to be differentially expressed in a metastatic pair of HNSCC cell lines: HSC3 (primary tumour, low expression) and HSC3-M3 (metastatic daughter line, high expression). Using this model, the role of LOX in hypoxia-induced metastasis was studied using invasion, migration and anoikis-resistance assays. LOX was also overexpressed and inhibited (using active site inhibitor BAPN) to determine LOX-specificity. Currently, we are investigating LOX as an inducer of RR by clonogenic assays, comet assay and γH2AX staining. Pathways including epithelial-to-mesenchymal transition (EMT) have so far been investigated as mechanisms. Next we will use bioinformatics to correlate follow up data from our original study and existing datasets, to LOX’s involvement in treatment failure and survival.

Results and discussions Our results have shown that high expression of LOX, endogenously in metastatic HSC3-M3 cells or by overexpression, can induce a metastatic phenotype resulting in increased invasion, migration and anoikis resistance. Inhibition of LOX reverses these effects, exaggerated in hypoxia where LOX is upregulated. LOX upregulation has also been linked to a change in expression/localisation of EMT regulators such as E-Cadherin and Vimentin. Early results show a potential link to RR. Ongoing studies aim to determine if this effect is a result of LOX’s enzymatic activity, or potential transcription factor functioning.

Conclusion This work will be the first to highlight LOX as an important biomarker not only in hypoxia-induced metastasis of HNSCC, but also in therapy resistance.

PO-200 PREVENTION OF MELANOMA BRAIN COLONISATION BY INHIBITING CYTOKINES SECRETION FROM ACTIVATED ASTROCYTES

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Introduction The brain microenvironment consists of various cell types such as astrocytes, microglia, neurons and brain endothelial cells. It sustains the normal brain functions and the structures of the capillary walls that form the blood brain barrier (BBB). During tumour progression, malignant cells release vascular endothelial growth factor and other cytokines that increase the permeability of blood vessels allowing them to intravasate into the circulation. In addition, the cells can take advantage of the patho-physiology of the tumour angiogenic leaky blood vessels at distant organs and extravasate from them at inflamed sites. Such activated site can be the brain parenchyma, which may become a pro-inflammatory metastatic niche. Although little is known about the precise mechanisms and the factors that contribute to the brain metastatic process, there is increasing evidence that astrocytes are involved by mediating a neuroinflammation. They respond by releasing pro-inflammatory cytokines that compromise the integrity of the BBB, increasing the permeability of the capillary endothelium and preparing a metastatic niche for melanoma colonisation.

Material and methods We established three spontaneous melanoma brain metastasis mouse models that present a pro-inflammatory microenvironment and a pro-metastatic niche preceding the colonisation of melanoma to the brain. siRNA targeting specific chemokines, neutralizing antibody, or a CRISPR/Cas9 system were used to downregulate the expression and secretion of chemokines for further in vitro studies.

Results and discussions We identified MCP-1, a pro-inflammatory chemokine, implicated in the paracrine interaction between melanoma cells and the brain. The positive staining for GFAP and MCP-1 in the tumour area suggests a cooperation between melanoma and the tumour microenvironment, in which MCP-1 plays a pro-tumorigenic role during cell migration. Astrocytes-secreted MCP-1 expression levels increase when co-cultured with melanoma cells or their conditioned media. Downregulation of MCP-1 leads to decreased migration of melanoma cells in vitro. Moreover, neutralising antibody-targeting MCP-1 reduces melanoma invasion in matrigel towards sprouting aortas.

Conclusion Activated astrocytes promote the migration of circulating melanoma cells to the brain by increased secretion of pro-inflammatory cytokines, such as MCP-1. Consequently, investigating the changes in melanoma and astrocytes gene-expression could be the key for interfering and preventing melanoma colonisation to the brain.

PO-201 NF1 INHIBITS GLIOMA CELLS INVASION AND REVERTS MESENCHYMAL TRANSITION

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Introduction Neurofibromin (NF1), a tumour suppressor and RAS-GTPase activating protein, is one of the highly mutated genes in cancer. Dysregulated NF1 expression promotes tumorigenesis, impairs learning and memory and affects neural development. Loss of NF1 expression is observed in approximately 13% of all glioma and is associated with increased malignancy. Additionally, NF1-loss increases glioma stem cells self-renewal, heightens cell invasion, and promotes
mesenchymal transition resulting in enhanced tumour aggressiveness. However, aside from its RAS-GAP function, it is not clear how NF1 mitigate cell invasion and mesenchymal transition. Herein, we show that the leucine-rich domain (LRD) of NF1 plays a role in cell invasion.

**Material and methods** Patient-derived glioma cells was used in this study. Immunohistochemistry staining was used for assessing proneural and mesenchymal markers expression.

**Results and discussions** Exogenous expression of LRD in NF1 knockdown glioma cells inhibited cell invasion *in vitro* and *in vivo*. Using immunohistochemistry staining for cancer stem cell markers associated with proneural (Sox2) and mesenchymal (CD44 and vimentin) subtype of glioblastoma, we found high Sox2 but low vimentin expression in LRD-expressing tumour when compared with the NF1-knockdown tumour.

**Conclusion** Our data suggests that LRD may play a role in reverting mesenchymal GBM to a less invasive and therapy-sensitive subtype.

**PO-202 THE ROLE OF RHOG IN MIGRATION AND INVASION OF ASTROCYTOMA CELLS**

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**Introduction** Metastasis is the movement of a tumour from its original site to a secondary site. The ability of cancer cells to metastasize depends on their motility and actin-rich membrane protrusions. These protrusions and motility are regulated by Rho GTPases, including RhoG. RhoG shares a lot of homology with Rac1 and was found to be overexpressed in many tumour tissues.

**Material and methods** In this paper, we studied the role of RhoG in migration and invasion of a brain tumour cell line. In order to delineate the role of RhoG, we knocked it down using siRNA. We studied its effect on migration by time lapse analysis. We also observed its effect on adhesion using an adhesion assay and staining the cells for vinculin.

**Results and discussions** We found that knocking down RhoG, using siRNA, 2D random migration of SNB19 cells significantly decreased. RhoG was also found to positively regulate cell invasion and cell adhesion. This suggested an effect of RhoG on Rac. Indeed, when RhoG was knocked down, Rac activation dramatically decreased in a pull down assay. Cell adhesion phenotype showed a complete lack of focal contacts in the RhoG KnDn cells. This was mimicked in the Rac KnDn cells. This was mimicked in the Rac KnDn cells. This suggested an effect of RhoG on Rac.

**Conclusion** This study shows that, in glioblastoma cells, RhoG positively regulates cell adhesion, migration and invasion through the activation of Rac.

**PO-203 PEROXIREDOXIN 5 IN COLON CANCER PROMOTES EPITHELIAL-MESENCHYMAL TRANSITION**

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**Introduction** Globally, colorectal cancer (CRC) is common cause of cancer-related deaths. The high mortality rate of patients with colon cancer is due to cancer cell invasion and metastasis. Initiation of the epithelial-to-mesenchymal transition (EMT) is essential for the tumorigenesis. Peroxiredoxins (PRX1-6) have been reported to be overexpressed in various tumour tissues, and involved to be responsible for tumour progression. However, the exact role of PRX5 in colon cancer remains to be investigated enhancing proliferation and promoting EMT properties.

**Material and methods** In this study, we constructed stably overexpressing PRX5 and suppressed PRX5 expression in CRC cells.

**Results and discussions** Our results revealed that PRX5 overexpression significantly enhanced CRC cell proliferation, migration, and invasion. On the other hand, PRX5 suppression markedly inhibited these EMT properties. PRX5 was also demonstrated to regulate the expression of two hallmark EMT proteins, E-cadherin and Vimentin, and the EMT-inducing transcription factors, Snail and Slug. Moreover, in the xenograft mouse model, showed that PRX5 overexpression enhances tumour growth of CRC cells. Thus, our findings first provide evidence in CRC that PRX5 promotes EMT properties by inducing the expression of EMT-inducing transcription factors.

**Conclusion** Therefore, PRX5 can be used as a predictive biomarker and serves as a putative therapeutic target for the development of clinical treatments for human CRC.