Chemotherapy induces PD-L1 in esophageal squamous cell carcinoma

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Introduction
Esophageal squamous cell carcinoma (ESCC) is one of the most aggressive and lethal cancers. Chemoresistance is a major obstacle in effective treatment for ESCC patients. Programmed death ligand-1 (PD-L1) is an immunoregulatory protein that is overexpressed in various cancers. PD-L1 up-regulation contributes to chemoresistance in several cancers, but little is known with respect to changes associated with chemotherapy treatment in ESCC.

Material and methods
A tissue microarray consisting of 84 ESCC tumours from Chinese patients was used to determine the PD-L1 expression and its correlation with clinicopathological parameters. Immunohistochemical (IHC) staining was performed with PD-L1 antibody and the staining intensity was scored. Two ESCC cell lines, KYSE150 and SLMT, were used. Cells were treated with either 5-Flurouracil plus cisplatin or carboplatin plus paclitaxel, which are the common regimens used for ESCC patients. The regulation of PD-L1 expression by the EGFR pathway and ERK pathway was studied using Erlotinib (EGFR inhibitor) and AZD6244 (MEK inhibitor). For the in vitro studies, an esophageal orthotopic mouse model was used. KYSE150 cells were injected into the mouse oesophagus. Mice were administered with 5-Flurouracil plus cisplatin or carboplatin plus paclitaxel by intraperitoneal injection. The change in PD-L1 expression was then evaluated by Western blotting and IHC staining.

Results and discussions
The expression frequency of PD-L1 in Chinese ESCC patients was 21% (18/84) and the patients with positive PD-L1 staining were associated with later stage (stages III and IV) of the disease. Also, high PD-L1 expression was associated with lymph node metastasis. Both in vitro and in vivo studies demonstrate that the level of PD-L1 expression increased after the treatment with 5-Flurouracil plus cisplatin or carboplatin plus paclitaxel. In vitro study shows that the elevated PD-L1 level was sustained even after the drugs were removed. By using pathway inhibitors, we demonstrate the increase in PD-L1 expression in response to chemotherapy was regulated by the EGFR pathway and its downstream ERK pathway, as the PD-L1 level attenuated when Erlotinib or AZD6244 was added.

Conclusion
PD-L1 expression was increased following treatment with chemotherapy in ESCC cell lines, suggesting that combining chemotherapy with PD-L1 blockade may improve treatment in ESCC patients.

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Differential effects of immune activity on melanoma cell subtypes

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Introduction
Glioblastoma is the most common and lethal type of brain cancer with a median survival of under fifteen months. It is a highly angiogenic tumour exhibiting an extremely invasive nature. It is well-known that the brain microenvironment plays a crucial role in glioblastoma progression although the large multitude of interactions between the cancer cells and their microenvironment are yet to be fully unravelled. Astrocytes are the most abundant glial cells in the brain and have been shown to be involved in many types of brain pathologies as well as metastatic colonisation in the brain. Hence, we investigated the influence of astrocytes on the migratory and infiltrative abilities of glioblastoma cells.

Material and methods
In order to evaluate the influence of brain microenvironment on glioblastoma progression we used co-culture proliferation and migration assays as well immunohistochemistry analysis of our mouse models and patient derived samples. Using protein profiler we assessed the level of cytokines secreted following interaction of glioblastoma cells and their microenvironment.

Results and discussions
Using in vitro and ex vivo assays, we found that in the presence of either astrocytes or their conditioned media, the migration rate of glioblastoma cells is significantly increased. Microglia are macrophages-like cells which possess antigen-presenting and phagocytic abilities that serve as the brain immune system. In a co-culture proliferation assay, we observed that microglia increased glioblastoma cells proliferation at a concentration-dependent manner. Furthermore, co-culture of glioblastoma cells with either astrocytes, microglia or brain endothelial cells resulted in elevated levels of similar cytokines. Moreover, immunohistochemistry analysis of several brain tumours inoculated orthotopically in mice revealed an increased level of activated astrocytes and microglia and high microvessel density within the tumoursite.

Conclusion
Our findings indicate that the brain microenvironment facilitates glioblastoma proliferation and invasion by cytokines secretion paving the way for investigation of their use as targets for glioblastoma therapy.

Brain microenvironment-secreted cytokines facilitate glioblastoma proliferation and invasion

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Differential effects of immune activity on melanoma cell subtypes

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Introduction
The cytokines IFNγ and TNFα are abundantly expressed by cytotoxic T cells. These cytokines have distinct and overlapping downstream effects. In this study, we assessed the effects of IFNγ and TNFα on the expression of immune regulatory factors.

Material and methods
We analysed a large panel of melanoma cell lines, including BRAF mutant (n=11), NRAS mutant (n=10), BRAF and NRAS wild type (n=10) cutaneous melanomas, and GNAQ/11-mutant uveal melanomas (n=8). These cells were treated with TNFα or IFNγ for 48 hour and the expression of PD-L1, PD-L2, HLA-ABC and HLA-DR was analysed by flow cytometry.

Results and discussions
IFNγ showed consistently stronger induction of PD-L1, PD-L2, HLA-ABC and HLA-DR

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Brain microenvironment-secreted cytokines facilitate glioblastoma proliferation and invasion

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EFFECTS OF CYCLING HYPOXIA ON THE COMMUNICATION BETWEEN MACROPHAGES AND ENDOTHELIAL CELLS IN PROMOTING TUMOUR GROWTH AND METASTASIS

Abstracts

PO-369

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Introduction Tumours are not only composed of malignant cells. Indeed, blood vessels, composed of endothelial cells, are required for tumour growth. Interestingly, the induction of angiogenesis requires not only endothelial cells but also immune cells. Among them, tumour-associated macrophages (TAMs) make up to 50% of the tumour immune infiltrate. These TAMs are strong angiogenesis inducers and are in part responsible of the tumour inflammation. Two kinds of macrophages are detected in tumours: pro-inflammatory and anti-tumoral M1 macrophages and anti-inflammatory and protumoral M2 macrophages.

Hypoxia is another key feature of tumour microenvironment which induces angiogenesis and enhances tumour inflammation and metastasis. Two types of hypoxia occur in tumour: chronic hypoxia, which impacts cells too far from the blood vessels, and cycling hypoxia (CyH), which causes intermittent oxygenation of malignant and non-malignant cells.

We have studied the impact of CyH on macrophage polarization and activity. Secondly, the impact of CyH on the communication between macrophages and endothelial cells was investigated. Then, the impact of this dialogue on tumour growth and metastasis will be studied.

Material and methods THP-1 monocytes are differentiated into M0 macrophages by PMA. M0 macrophages are then polarised into M1 macrophages with LPS and IFNg (24 hour incubation) or into M2 macrophages with IL-4 and IL-13 (48 hour incubation). After exposing these macrophages to normoxia, chronic hypoxia or CyH, the expression and secretion of pro-inflammatory factors were studied by RT-qPCR and ELISA, respectively. To investigate the communication between macrophages and endothelial cells under CyH, endothelial cells were incubated with CyH-exposed macrophage-conditioned-medium and the expression of endothelial cell activation markers was analysed by RT-qPCR.

Results and discussions CyH induced a pro-inflammatory phenotype in M0 macrophages and enhanced the pro-inflammatory phenotype of M1 macrophages. Indeed, an increased expression of TNFa, IL-6 and IL-1β was observed. These effects depended on NF-kB activation since IKK inhibition prevented these effects. The conditioned-media of M0, M1 and M2 macrophages exposed to CyH induced endothelial cell activation as observed by an increased IL-6, IL-8 and ICAM-1 expression.

Conclusion CyH induced a pro-inflammatory phenotype in M0 and M1 macrophages via NF-kB activation. The M0, M1 and M2 macrophages exposed to cycling hypoxia induced endothelial cell activation by a secreted molecule that needs to be identified.

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A CO-CULTURE ASSAY SYSTEM USING ENGINEERED ANTI-CD3 TUMOUR CELLS TO ASSESS TUMOUR CELL SENSITIVITY TO CD8+ T CELL KILLING

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Introduction Immunotherapy has had a significant impact on the landscape of cancer treatment. However, with only a 20–30% response rate, identifying novel tumour intrinsic mechanisms of resistance is key to advancing treatment success. Activation of CD8+ T cells is crucial to tumour cell lysis. Therefore, cytotoxic assays that measure CD8+ T cell responses against tumours, are crucial for understanding therapeutic outcomes.

Material and methods We have developed an in vitro system in which we have engineered tumour cells to express single-chain (scFv) anti-CD3 (clone OKT3) which provides signal 1 to CD8+ T cells, in a co-culture assay. This system allows us to understand the relative contributions of MHC-independent mechanisms to T-cell killing of a panel of tumour cell lines, in parallel. As a model, we have transduced the EGFR mutant non-small cell lung cancer (NSCLC) cell lines PC-9, NCH-H1975 and NCI-H3255 to stably express anti-CD3.

Results and discussions Immune checkpoint blockade yields a clinical response in a subset of NSCLC patients. However, EGFR mutant NSCLC patients show less favourable responses. This assay allows us to investigate how EGFR genotype might...