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Poster Presentations

P001

IFN-Λ4 HAS A POTENT ANTIPROLIFERATIVE ACTIVITY IN B-CELL LYMPHOMA AND HEPATOMA CELL LINES

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Introduction: Type-I interferons (IFNs) have been used to treat several types of cancers including follicular lymphoma, hairy cell leukemia and chronic myeloid leukemia. Treatment with type-I IFNs is however associated with significant toxic side effects due to the ubiquitous expression of type-I IFN receptors. Type-III IFNs elicit similar cellular responses as type-I IFNs, but restricted expression of type-III receptors may limit potential side effects of treatment with type-III IFNs. A number of studies are now exploring the use of type-III IFNs, specifically IFN-λ3, as anticancer agents. IFN-λ4 is a newly discovered type-III IFN that is genetically regulated and produced in a subset of individuals. Previously, we showed that IFN-λ4 inhibits proliferation in a hepatoma cell line, however its anticancer potential has not been explored in comparison to other type-III IFNs.

Methods: We developed stable human hepatoma (HepG2) and B-cell lymphoma (Raji) cell lines, engineered to express either IFN-λ4-GFP or IFN-λ3-GFP upon induction by doxycycline. We compared antiproliferative activity of IFN-λ3 and IFN-λ4 by measuring BRDU incorporation before and after induction of IFN-λ expression. We also compared cell viability, apoptosis, and changes in cell cycle between IFN-λ3 and IFN-λ4 expressing cell lines. RNAseq and pathway analysis were used to explore the antiproliferative effect induced by IFN-λ4.

Results: We show that IFN-λ4 has higher antiproliferative activity compared to IFN-λ3 in Raji and HepG2 cell lines and this can be explained by inhibition of the cell cycle. IFN-λ4 expressing cell lines showed increased apoptotic cell death compared to IFN-λ3 expressing cell lines. We also show that antiproliferative activity of IFN-λ4 may be a result of inhibition of the MAPK pathway.

Conclusion: This study demonstrates that IFN-λ4 has a more potent antiproliferative activity than IFN-λ3 in hepatoma and B cell lymphoma cell lines and the anticancer potential of IFN-λ4 should be further explored.

Disclosure of Interest: None declared.

P002

HCV CORE PROTEIN TRIGGERS IL-1B PRODUCTION VIA THE NLRP3 INFLAMMASOME

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Introduction: Interleukin-1β (IL-1β) is a potent proinflammatory cytokine that is implicated both in acute and chronic viral infection. Persistent hepatic inflammation is the hallmark of hepatitis C virus (HCV) infection wherein it serves as a platform for progressive liver injury and onset of liver cancer.

Methods: We examined the role of IL-1β role in HCV-induced hepatic inflammation by first assessing the transcriptional profile of chronic HCV patient liver by next-gen sequencing (RNAseq) staged by inflammation and fibrosis score.

Results: RNAseq and bioinformatic analyses revealed that IL-1β production associates with liver disease linked with expression of a range of IL-1β-responsive inflammatory genes known to support tissue remodeling, leukocyte homing and activation, and proinflammatory cytokine production and amplification. Further, we found that hepatic macrophages, known as Kupffer cells, are a major source of liver IL-1β. Typically, IL-1β production is a tightly regulated pathway requiring a priming step to trigger IL-1β transcription and an activating signal to promote mature IL-1β processing and release. We found that the HCV virion is efficiently phagocytosed by the Kupffer cell and human THP1 macrophage cell line to drive signaling through TLR7/MyD88 and NLRP3 inflammasome. This process triggers IL-1β expression and mature IL-1β protein release, respectively. We have now identified the specific HCV-virion component, HCV core protein, as a driver of NLRP3 inflammasome activation. HCV core protein modulates many cellular events such as reactive oxygen species production and calcium signaling, all of which have been shown to activate the NLRP3 inflammasome pathway. We found patient-derived core protein, isolated from patients with HCV infection, can stimulate NLRP3 inflammasome activation and trigger the release of IL-1β.

Conclusion: Our studies reveal HCV virion-associated core protein as the trigger of viral-induced NLRP3 inflammasome activation within hepatic macrophages. Our observations define the HCV-Kupffer cell interaction as a central node directing hepatic inflammation underlying HCV pathogenesis.

Disclosure of Interest: None declared.

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P003

REGULATION OF THE CCL2-CCR2 AXIS THROUGH THE JAK-STAT PATHWAY AND ROLE IN CANCER METASTASIS

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Introduction: Chemokine receptors and their ligands are expressed on several tumour and cancer cells. Their expression has been correlated with migration and metastasis of cancer/tumour cells. CCR2 is expressed under varied immune conditions in T cells, B cells, natural killer cells, basophils, and dendritic cells. Furthermore, CCR2 is expressed in cancer tissues and upregulation correlates with advanced cancer/tumour, metastasis and relapse. CCR2 is regulated at the genomic level by several transcription factors which upon binding to the promoter of the gene, leads to either an upregulation or downregulation of the gene. One of the several proteins and pathways is the JAK-STAT3/5 pathway.
The JAK-STAT3/5 pathways play a crucial role in cell differentiation, proliferation and thus has a critical role in cancers/tumours. The JAK-STAT3/5 pathway is regulated by cytokines including IL-2, IL-15 and IL-6 amongst several others. IL-2 has been well studied in cancer immunotherapy trials and current immunotherapy trials are also taking place with IL-15 in man. However, the precise mechanism of action and the cascade of proteins and events involved are not completely understood. This study, aims to investigate the regulation of the JAK-STAT pathway by IL-2 and IL-15 and its role in CCR2 mediated cancer metastasis in prostate cancer.

Methods: Preliminary studies on the JAK-STAT5 regulated genes using quantitative real time PCR and quantitative PCR in concert with bioinformatic analysis on publicly available databases have identified CCR2 as an IL-2 regulated STAT5 target gene.
Interestingly, preliminary studies on cancer databases have shown activation of CCR2 through several other hormones and growth factors.

Results: The STAT5 binding site identified within the CCR2 gene, mapped to the promoter of the gene and its co-ordinates on the UCSC genome browser (hg38) are chr3:46,351,020-46,351,025 and chr3:463,520,53-463,520,58. The two sites have differential binding of the STAT5 complex.
The CCR2 gene is regulated by IL-2 in T cells and public databases have shown an upregulation in solid cancers. Its role in prostate cancer is under investigation.

Conclusion: CCR2 plays an important role in cancer metastasis. Prostate cancer is the most common cancer in men and unfortunately, tumour cells may develop resistance to therapy and become metastatic. This then becomes a major obstacle, limiting the success in prostate cancer treatment. The complicated crosstalk, both genomic and nongenomic, between transcription factors, immune cells and cytokines/growth factors is considered to be a crucial factor contributing to metastasis. Although several mechanisms have been proposed, so far none of them can be defined as the rationale behind the phenomena of metastasis. This study, is in progress to delineate the role of the IL-2/IL-15 regulated JAK-STAT5 pathway in regulating the CCR2 gene and thus the CCL2-CCR2 axis.

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P004

LOSS OF IRF5 INDUCES SPONTANEOUS MAMMARY TUMORIGENESIS

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Introduction: Despite significant research efforts, breast cancer is still the most common malignancy in women and the most common cause of cancer death worldwide. A major challenge in breast cancer treatment is disease heterogeneity that is contributed by many factors including intrinsic cell factors, microenvironment, angiogenesis and tumor-specific immune responses. Interferon regulatory factor 5 (IRF5) is a transcription factor that controls inflammatory and immune responses. Analysis of over 3000 human breast cancer tissues revealed that high expression of *IRF5* correlates with increased survival and lower incidence of metastasis, whereas the lower quartile of *IRF5* expression is a significant marker of poor prognosis for recurrence-free survival. These data support a tumor suppressor role for IRF5. The main purpose of this study was to generate a murine model of spontaneous mammary tumorigenesis in order to determine whether loss of IRF5, as found in human breast tumor tissue, contributes to disease onset and/or progression.

Methods: *Irf5* knockout (ko) mice were backcrossed to BALB/C mice to obtain an F10 generation of *Irf5*ko Balb/c mice that retain wild-type Dock2 expression. Wild-type BALB/c mice are susceptible to spontaneous mammary tumorigenesis at a low incidence rate. The effect(s) of *Irf5* deficiency on normal mammary gland development and mammary tumorigenesis was examined.

Results: In mammary glands harvested from one-year-old female virgin ko mice (n = 20), we found an increased incidence (15%) of mammary carcinoma *in situ* as compared to their wild-type littermates (5%; n = 20). Of interest, the number of tumor bearing mice was significantly higher (38%) in retired ko female breeders (n = 8); however, there were no abnormalities detected in one-year-old male ko virgins or breeders (n = 6). Cellular and molecular analysis of female ko and wild-type littermates revealed that *Irf5* deficiency enhanced mammary epithelial cell proliferation as indicated by an increased number of Ki67 expressing cells, and resulted in abnormal hyperbranching, which may contribute to mammary tumorigenesis. *Irf5* ko breeders also showed impaired mammary gland involution after weaning, which has been shown to facilitate mammary tumor formation in humans.

Conclusion: Taken together, these findings demonstrate that IRF5 is a gender-specific tumor suppressor in breast cancer. In this ongoing study, we are further investigating the intrinsic and extrinsic