MODULATION OF TLR3 PROTEIN IN RESPONSE TO RADIATION IN SQUAMOUS CELL LUNG CARCINOMA

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Background Squamous cell lung cancer (SCLC) is the second most common type of lung cancer. Treatment is complicated due to the lack of mutated molecular targets.¹ Radiotherapy (RT) is commonly used to treat SCLC, but relapse and tumor progression are common. The combination of immunotherapy (IT) with RT can enhance the effect observed with RT alone.² Effective combination of IT and RT requires an understanding of the pathways that synergize to enhance tumor cell kill in SCLC. Our lab has identified Toll-like receptor 3 (TLR3) as a molecule that is regulated by RT and can be targeted with IT. Toll-like receptors serve a crucial role against tumor cells by activating innate and adaptive immune responses that boost antitumor immunity.³⁻⁴ TLR3 is the only receptor whose molecular mechanism functions independent of MyD88, leading to NF- κ B mediated apoptosis.⁵ We hypothesized that increased TLR3 expression would be associated with improved response to RT. We further hypothesized that RT can downregulate TLR3 and that this effect can be reversed with TLR3 agonists leading to enhanced tumor antigen recognition. We aim to use this data to formulate further studies using combined RT and IT.

Methods Mouse (KLN205) and human (SW900) squamous cell carcinoma (SCC) cell lines were used to study the effect of radiation on TLR3 expression. Irradiation was performed using the gammacell 3000 elan irradiator. Cells were irradiated with 0, 5, 10 and 20 Gy. Protein extraction was performed 48 and 72 hours after RT. Protein extracts were analyzed by Western Blot. Further, TLR3 mRNA expression and 5-year overall survival of SCLC patients was obtained from public databases. Kaplan-Meier method was used to correlate between TLR3 mRNA expression and survival.

Results In vitro studies and western blot analysis demonstrated a decrease of TLR3 expression in response to increasing doses of radiation. This observation was consistent in mouse and human SCC cell lines. In silico analysis of SCLC patients who received RT showed that increased TLR3 mRNA expression was associated with improved overall survival and disease-free survival.

Conclusions Our findings point to an important role for TLR3 in SCLC. Combining RT with TLR3 agonists may enhance the tumor response to RT. Several complementary experiments are underway in our lab to use the TLR3 agonist, Poly I:C, which will allow a better understanding of the effect of RT on TLR3.

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COVID and immunotherapy

DEEP IMMUNE PROFILING OF SARS-COV-2 ASSOCIATED IMMUNE MICROENVIRONMENT IN CANCER TISSUES FROM RECOVERED COVID-19 PATIENTS

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Background Persistence of SARS-CoV-2 virus particles in recovered COVID-19 patients remains a challenge as we continue to fight the ongoing pandemic. For instance, despite three negative consecutive nasopharyngeal swab PCR tests, residual SARS-CoV-2 was reported in the lungs of a deceased patient.¹ Moreover, viral RNA could also be detected in rectal tissues that were obtained during incubation period.² To date, there is no data regarding residual viral particles present in tissues from recovered COVID-19 patients. Hereby, we reported our findings of SARS-CoV-2 viral antigen in liver tissues from a recovered COVID-19 patient. These findings raise concern for potential transmissibility in recovered individuals.

Methods A 49-year-old South Asian male diagnosed with COVID-19 in June 2020, with incidental discovery of hepatitis B virus (HBV)-associated R0 Grade 2 hepatocellular carcinoma (HCC), was consented for our study. He did not develop significant acute respiratory symptoms throughout the course of the disease. He underwent curative resection of HCC 85 days after being tested COVID-19 negative where his blood, normal tissue and tumour samples were obtained for further analysis (figure 1). We performed deep immunopathological profiling on the specimens using multiplex immunohistochemistry and 25-colour flow cytometry to study SARS-CoV-2-elicited immune response.

Abstract 825 Figure 1 Study design, methodology and brief summary of the findings

Blood, normal tissue and tumour samples were obtained from a 49-year-old South Asian male who was diagnosed with COVID-19 and hepatocellular carcinoma. Normal tissue and tumour samples were analysed with multiplex immunohistochemistry, while dissociated cells from blood and tissue samples were subjected to SARS-CoV-2 peptide stimulation and analysed with 25-colour flow cytometry. Multiplex immunohistochemistry detected SARS-CoV-2 proteins in both tumour and adjacent normal tissues, while flow cytometry identified distinct immune microenvironment involving memory-like T cells.

Abstracts

Results Multiplex immunohistochemistry detected SARS-CoV-2 nucleocapsid protein only in adjacent normal liver tissue but not within tumour core (figure 2). We also observed SARS-CoV-2 in some immune cells such as sinusoidal Kupffer cells (figure 2). Additionally, upon stimulation with SARS-CoV-2 peptides, we successfully elicited SARS-CoV-2-specific memory response which is distinct from the response upon challenge with HBV peptides. These findings were similar to our previous discovery in a patient with colorectal adenocarcinoma where we have shown viral antigen detection, validated with PCR to detect viral RNA, as well as the detection of SARS-CoV-2 memory-like T cells in situ (figure 2). Deep profiling of the samples is on-going with single-cell analysis and digital spatial profiling.

Conclusions We believe this is the first immune profiling report of the in situ tumour microenvironment in a cancer patient with COVID-19. Our findings demonstrated the presence of viral proteins in the liver despite negative swab test result and the ability to elicit ex vivo SARS-CoV-2-specific immune responses through peptide stimulation assays. We also detected same immune cell phenotypes in situ in the cancer

tissues. Taken together, we propose caution when handling tissues from patients who have a recent history of COVID-19, particularly during aerosol-generating procedures such as ultrasonic dissection surgery.

Ethics Approval This study was approved by Centralised Institutional Review Board of SingHealth, approval number 2019/2653.

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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826

RISK OF COVID-19 INFECTION AMONG PATIENTS RECEIVING IMMUNE CHECKPOINT INHIBITOR THERAPY: A TERTIARY CARE HOSPITAL SYSTEM COLLABORATION WITH THE MASSACHUSETTS DEPARTMENT OF HEALTH

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Background It is unclear whether treatment with immune checkpoint inhibitors (ICIs) is a risk factor for contracting COVID-19. We investigated whether patients prescribed ICIs were more or less likely to contract COVID-19 than matched controls in a collaboration between a large academic health-care system and the Massachusetts Department of Health.

Methods We performed a retrospective study of 1,577 cancer patients prescribed ICI and 26,241 matched controls. We included all patients infused with an ICI in the Mass General Brigham (MGB) network between 7/1/19 and 2/29/20 and

Abstract 825 Figure 2 Immunohistochemical staining of the SARS-CoV-2 nucleocapsid protein and immune profiling with 25-colour flow cytometry in normal colon and liver tissue a, Liver tissues were immunostained with SARS-CoV-2 nucleocapsid protein (NP), nuclei were counterstained with haematoxylin. Positive SARS-CoV-2 nucleocapsid staining in benign hepatocytes and sinusoidal Kupffer cells. Scale bar represents 50µm. b, Multiplex immunohistochemistry of normal liver tissue. From left to right, top to bottom: SARS-CoV-2 nucleocapsid (green), SARS-CoV-2 nucleocapsid (green) with CD14 (red), SARS-CoV-2 nucleocapsid (green) with CD68 (pink) and composite. Co-localisation were observed as shown by the white arrows. Scale bar represents 100µm. c, Colon tissues were immunostained with SARS-CoV-2 nucleocapsid protein, nuclei were counterstained with haematoxylin. Positive SARS-CoV-2 nucleocapsid staining in colonic crypts, with granular supranuclear cytoplasmic pattern. Scale bar represents 50µm. d, Multiplex immunohistochemistry of colon tissue. From left to right, top to bottom: DAPI (blue), CD3 (magenta), CD38 (green), granzyme B (yellow), interferon-gamma (red) and composite. Co-localisation was observed as shown by the white arrows. Scale bar represents 100µm, Magnification x200. e, Flow cytometry immune profiling of blood from colorectal cancer patient with COVID-19 following stimulation with SARS-CoV-2 peptides. Highlighted populations showed CD3 cells expressing CD38, supporting the CD3+ CD38+ co-localization findings observed in (c).

Abstract 826 Table 1 Multivariable logistic regression for patients treated with ICI with Odds Ratios for defining 'event' to be COVID-19 positivity

	OR	95% CI	p-value
ICI use	0.7	0.45 – 1.09	0.11
Age			
18–44	ref*	ref*	ref*
45–64	0.86	0.56 – 1.32	0.49
65–74	0.54	0.34 – 0.86	0.01
74+	0.97	0.61 – 1.56	0.91
Sex Female	1.08	0.91 – 1.28	0.39
Race and Ethnicity			
Non-Hispanic White	ref*	ref*	ref*
Non-Hispanic Asian or Pacific Islander	2.38	1.60 – 3.54	<0.001
Non-Hispanic Black	1.63	1.10 – 2.42	0.02
Non-Hispanic Other	1.77	1.08 – 2.93	0.03
Hispanic	0	0.00 – Inf	0.99
Unknown	1.76	1.06 – 2.92	0.03
CCI grade			
Mild (1–2)	ref*	ref*	ref*
Moderate (3–4)	3.01	1.45 – 6.28	0.00
Severe (≥5)	3.97	1.88 – 8.38	<0.001
Comorbidity			
Median income in \$1,000s	0.90	0.87 – 0.94	<0.001
COVID-19 town or county positivity rate per 100,000	1.22	1.15 – 1.30	<0.001