PATHOLOGY AND LABORATORY MEDICINE

RESEARCH DAY 2021

PLATFORM PRESENTATION ABSTRACTS

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Schulich MEDICINE & DENTISTRY

London Health Sciences Centre

Western

MORNING SCHEDULE

9:00 a.m. **Welcome and Opening Remarks**
Dr. Subrata Chakrabarti
Chair/Chief, Pathology and Laboratory Medicine, Schulich Medicine & Dentistry, Western University and London Health Science Centre

9:15 a.m. **Platform Presentations 1**
Jonathan Keow
Karissa French
Christopher Tran

10:00 a.m. Break

10:15 a.m. **Platform Presentations 2**
Alice Shin
Michael Roes
Darya Abdolmaleki

11:00 a.m. Break

11:15 a.m. **Keynote Address**
Dr. Michael Snyder
Stanford W. Ascherman Professor and Chair, Department of Genetics at Stanford University

AFTERNOON SCHEDULE

1:30 p.m. **Poster Presentations 1**
Concurrent session 1A: Applied Clinical Research 1
Concurrent session 1B: Cancer Biology 1
Concurrent session 1C: One Health

2:30 p.m. **Poster Presentations 2**
Concurrent session 2A: Bioinformatics and Data Science
Concurrent session 2B: Neuropathology
Concurrent session 2C: Cardiovascular Biology

3:30 p.m. **Platform Presentations 3**
Concurrent session 3A: Applied Clinical Research 2
Concurrent session 3B: Cancer Biology 2
Concurrent session 3C: Infection and Immunity, Special COVID Session

ORAL PRESENTATIONS

Time	First Name	Last Name	Title
9:15 a.m.	Jonathan	Keow	Digital analysis of p16 identifies a pulmonary fibrosis phenotype with distinct outcomes
9:30 a.m.	Karissa	French	A survey of clinician satisfaction with the hospital autopsy process
9:45 a.m.	Christopher	Tran	The COVID-19 pandemic: Lessons for healthcare service delivery from surgical pathology data
10:15 a.m.	Alice	Shin	F4/80+Ly6Chi macrophages contribute to colon cancer initiation in colitis
10:30 a.m.	Michael	Roes	HOXA9 promotes enzalutamide resistance in RB-p53 deficient prostate cancer
10:45 a.m.	Darya	Abdolmaleki	Influence of Glucocorticosteroids on T-helper 17 Phenotype Development

ORAL PRESENTATION 1

Presenter's Name: Keow, Jonathan

Additional Authors: Cecchini MJ, Zompatori M, Joseph MG, Mura M

Abstract Title: Digital analysis of p16 identifies a pulmonary fibrosis phenotype with distinct outcomes.

Introduction: Interstitial lung disease (ILD) encompasses a spectrum of conditions with distinct clinical and pathologic features. Idiopathic pulmonary fibrosis (IPF) is associated with increased expression of cyclin-dependent kinase inhibitors such as p16, and subsequent induction of cell cycle arrest, cellular senescence, and pro-fibrotic gene expression. Given that IPF may be linked to abnormal cellular senescence, we sought to link p16-expression to a diagnosis of IPF or other fibrosing interstitial lung diseases (ILDs), radiographic pattern, senescent foci-specific gene expression, antifibrotic therapy response, and lung transplant (LTx)-free survival.

Methods: Eighty-six cases of fibrosing ILD were identified with surgical lung biopsy (2003-2018). p16 immunohistochemistry was performed on representative sections with the most active fibrosis. p16-positive senescent foci (defined as a loose collection of p16-positive fibroblasts with an overlying p16-positive epithelium) were identified and quantified per 100 mm2 of lung tissue. Cases were scored as p16-low (≤ 2.1 foci per 100 mm2) or p16-high (> 2.1 foci per 100 mm2). Fibroblastic foci, fibrotic and normal areas were characterized with digital spatial profiling using in situ RNA expression analysis.

Results: The presence of any p16-positive senescent foci was specific (92%) for the diagnosis of UIP. There were between 0 and 23 p16-positive senescent foci per slide. Forty-three (50%) cases expressed any level of p16 and 22 (26%) cases expressed high levels of p16. There was no relationship between the pre-biopsy HRCT pattern and p16 expression. There was increased expression of senescence and matrix remodeling genes within p16-positive foci of senescence Cases with high p16 expression had shorter LTx-free survival. However, antifibrotic therapy was protective with no difference in outcome based on p16 status.

Discussion: We demonstrated the clinical applicability for standardizing quantification of p16-positive senescent foci. This method identifies an IPF phenotype associated with upregulation of senescence-associated and matrix remodeling gene expression. These patients have reduced LTx-free survival, but have a robust response to antifibrotic therapies.

ORAL PRESENTATION 2

Presenter's Name: French, Karissa

Additional Authors: Jacques R

Abstract Title: A survey of clinician satisfaction with the hospital autopsy process

Introduction: When there is a death of an inpatient or a stillbirth, the clinicians in agreement with the family have the option to pursue a hospital authorized autopsy. Currently, there is little communication between the referring clinician and the consulting pathology service outside of the autopsy reports. We do not have a measure of how our service is meeting the needs of our clinicians. This quality improvement project aims to elucidate the level of satisfaction with our hospital autopsy service and identify areas of potential improvement.

Methods: A 17 question incentivized electronic survey composed of mixed linear, multiple choice, and long answer responses. The participants were selected with purposive sampling, choosing the four subspecialties which account for over half of referrals. The total number of trainees invited was 122 and consisted of residents and fellows from: cardiac surgery, obstetrics, genetics, and residents from all subspecialties whom have rotated through critical care units within the six months prior to this study. All 76 consultants from these four services were also invited to participate. Questions were crafted around two main domains: opinions and thoughts about autopsy in general and satisfaction with the autopsy service.

Results: Preliminary response rates: 7.9% of consultants, 12.3% of residents, with a combined total response of 10.6%. Clinicians strongly agreed that the hospital autopsy played an important role in patient care and quality assurance. When asked the overall satisfaction with the hospital autopsy service the result was positive, lowest scores were in verbal communication and turn around time.

ORAL PRESENTATION 3

Presenter's Name: Tran, Christopher C

Additional Authors: Kadour M, Cecchini MJ, Leslie KA, Driman DK

Abstract Title: The COVID-19 pandemic: Lessons for healthcare service delivery from surgical pathology data

Introduction: The COVID-19 pandemic has had a significant impact on the delivery of healthcare services. Notably, the Ontario-wide lockdown from March to June 2020 resulted in an estimated backlog of 148,364 surgeries. Although these reductions raised concerns regarding gaps in patient care, there is a paucity of data on how these changes affected pathology services. In this study, we evaluated the impact of the COVID-19 lockdown on surgical pathology volumes, and used pathology data to identify how changes in the pathology lab may reflect healthcare delivery at the systems level.

Methods: A descriptive analysis was performed using 2019-2020 summary surgical pathology data from the London Health Sciences Centre and St. Joseph's Health Care, with a focus on the lockdown period between March and June 2020. Monthly count data were collected for total accessioned specimens, specimen type (biopsy or resection), and specimen site. Cancer Care Ontario (CCO) submissions, representing newly staged cancer cases, were collected for each month and disease site. The relative changes in volumes between 2019 and 2020 were calculated.

Results: In April 2020, during the peak of the lockdown, the total number of accessioned specimens decreased by 67.5% compared to April 2019 (2,820 vs 8,668), while resection specimens decreased by 51.4% (524 vs 1,078). The number of CCO submissions decreased by 30.5% (169 vs 243). In the quarterly period from April to June 2020, the gap compared to 2019 decreased as surgeries resumed. Total specimens were reduced by 47.4% (13,842 vs 26,303), compared to 30.9% for resections (2,304 vs 3,335) and 11.3% for CCO submissions (643 vs 725). The reductions in CCO submissions varied between disease sites. While volumes were comparable for breast cancer (170 vs 171) and colon cancer (73 vs 76), there were greater drops of 71.6% (19 vs 67) and 53.2% (29 vs 62) for lung and prostate cancers, respectively. Biopsy volumes were variably decreased between disease sites, with colorectal biopsies down 63.7% (2,013 vs 5,553), compared to 24.4% for breast biopsies (374 vs 495) and 23.9% for prostate biopsies (730 vs 959).

Discussion: Surgical pathology volumes were expectedly reduced during the COVID-19 lockdown, but variable reductions were observed between specimen types and disease sites. CCO submissions, representing newly staged oncologic resections, were not as severely affected; this may reflect prioritization of oncologic surgeries. Differences in CCO submissions between disease sites may be due to triaging of cases based on patient, clinical, safety and resource factors. Unlike resections, biopsies may be performed in a range of clinical settings including clinics, operating rooms, and endoscopy and radiology suites. The observed changes in biopsy volumes may have resulted from restrictions on screening tests and workup for non-acute indications. Overall, our findings highlight potential gaps in care; resource planning to address such gaps should ensure patients have fair and equitable access to health care resources. The use of surgical pathology data can used to better understand healthcare service delivery at a broader level, and may be valuable in guiding resource decisions.

ORAL PRESENTATION 4

Presenter's Name: Shin, Alice

Additional Authors: Shin AE, Good HJ, Tesfagiorgis Y, Zhang L, Kerfoot SM, Sherman PM, Wang TC, Howlett CJ, Asfaha S

Abstract Title: F4/80+Ly6Chi macrophages contribute to colon cancer initiation in colitis

Introduction: Colorectal cancer is the second leading cause of cancer death with a major risk factor being chronic inflammation. Indeed, patients with inflammatory bowel disease (IBD) are at increased risk of colon cancer; however, the mechanism by which colitis leads to cancer is not known. Our previous studies showed that the type of colonic injury, rather than the simple presence of inflammation, is most important for colonic tumorigenesis. Thus, we aim to compare various colitis models with respect to their ability to initiate tumor formation. We hypothesize that dextran sodium sulfate (DSS)-induced colitis leads to the infiltration of specific immune cell populations that contribute to colonic tumorigenesis.

Methods: Following injection of the carcinogen azoxymethane (AOM), mice were treated with the colitis-inducing agents DSS, trinitrobenzene sulfonic acid (TNBS), oxazolone, Citrobacter rodentium, or Doxorubicin (Doxo), and colonic tumorigenesis were compared. Flow cytometry analysis of immune cells in the colonic tissue was performed to better characterize the various models of colitis. Additionally, the mRNA transcriptional profile of patients with ulcerative colitis was compared to that of the various mouse models of colitis.

Results: Treatment with DSS, TNBS, oxazolone, C. rodentium, or Doxo induced colonic inflammation as detected by increased myeloperoxidase (MPO) activity and histology assessment. DSS administration led to colonic tumors, whereas TNBS, oxazolone, C. rodentium, and Doxo did not lead to tumorigenesis even up to 52 weeks following repeated colitis induction. Flow cytometry analysis of immune cells in the colonic tissue revealed no difference in the number of T and B cells among mice treated with the different colitis inducing agents. In contrast, we detected significantly increased levels of Ly6G+ neutrophils and F4/80+Ly6Chi macrophages in the DSS-treated mice versus mice with colitis induced by the other colitogens. Additionally, mRNA and protein array analyses of the colonic tissue and analysis of the RNA-seq data from 206 UC patients (GSE109142) revealed increased expression of genes associated with myeloid cells. Antibody-mediated depletion of Ly6G+ neutrophils did not affect tumorigenesis, whereas clodronate liposome-mediated depletion of F4/80+Ly6Chi macrophages significantly reduced the number of colonic tumors.

Discussion: Our data suggest that infiltration of F4/80+Ly6Chi macrophages unique to DSS-induced colitis leads to colonic tumor formation. These findings demonstrate that specific myeloid cells are critical to the initiation of colitis-associated cancer.

ORAL PRESENTATION 5

Presenter's Name: Roes, Michael

Additional Authors: Dick FA

Abstract Title: HOXA9 promotes enzalutamide resistance in RB-p53 deficient prostate cancer

Introduction: Molecularly targeted cancer therapies are effective in improving patient outcomes while reducing harmful side effects and act by inhibiting molecules that cancer cells depend on for proliferation. However, resistance to these therapies is common. Castration resistant prostate cancer (CRPC) cells can acquire resistance to the anti-androgen enzalutamide (EZ) by switching lineages from an adenocarcinoma to a neuroendocrine (NE) cell type that no longer depends on androgen signaling for proliferation. Cancer genomic studies have identified frequent loss of the retinoblastoma (RB1) gene as a defining feature of these cells. Recently, the RB protein has been implicated in functions beyond classic cell cycle control, in which this protein maintains epigenome structure and integrity. Mutations in RB1 create a state of epigenetic instability, accompanied by increased expression of pluripotency factors. While mechanistic insight into epithelial de-differentiation is limited, RB1 loss has been identified as the most significant factor in determining poor survival and the shortest time on therapy for CRPC patients. This is remarkable because RB1 is often co-deleted with TP53, but p53 disruption does not have this impact on therapeutic resistance. In this study, I hypothesize that mutations in RB1 misregulate stemness and differentiation pathways that result in an increased propensity to transdifferentiate and acquire EZ resistance in prostate cancer.

Methods and Results: A genome-wide CRISPR knockout (KO) screen was performed in prostatic adenocarcinoma LNCaP cells. Pools of KO cells were treated with either EZ or DMSO and then analyzed by next generation sequencing to identify gene mutations that confer either increased resistance or sensitivity to EZ. Gene ontology (GO) analysis identified genes related to stem cell differentiation, such as HOXA9, to be de-enriched in the EZ treatment pools, suggesting that a stem cell like state is likely important for LNCaP cell survival during EZ treatment. As expected, RB1 mutation was highly enriched in the treatment pools, confirming that RB loss promotes EZ resistance. Since combined RB-p53 loss is known to promote NE transdifferentiation and EZ resistance in prostate cancer, RB-p53 double knockout (DKO) LNCaP cells were generated using CRISPR-Cas9. DKO cells express increased mRNA levels of the NE marker NSE, and form distinct colonies following chronic EZ treatment, compared with LNCaP parental cells. RNA-sequencing of DKO and LNCaP parental cells, followed by GO analysis, revealed differentially expressed genes related to neuronal processes and cell differentiation. In particular, the stemness factor HOXA9 was significantly upregulated in DKO cells. To investigate the functional link of HOXA9 in EZ sensitivity and RB-p53 dependency, mutations in HOXA9 that lead to its overexpression were generated in RB-p53 DKO cells. HOXA9 overexpressing cells were highly resistant to EZ over a 6-day acute EZ treatment, compared with DKO and LNCaP parental cells. Furthermore, HOXA9 overexpression caused the formation of significantly more and larger colonies following chronic EZ treatment, compared with parental cells.

Discussion: Overall, these results suggest that HOXA9 has important roles in promoting EZ resistance in RB-p53 deficient prostate cancer cells. Furthermore, HOXA9 inhibition may have therapeutic benefits for the treatment of EZ-resistant prostate cancers.

ORAL PRESENTATION 6

Presenter's Name: Abdolmaleki, Darya

Additional Authors: Solomon L, Raveendraraj J, Liang O, Karunagaran S

Abstract Title: Influence of Glucocorticosteroids on T-helper 17 Phenotype Development

Introduction: Asthma affects more than 300 million people worldwide, causing great morbidity to the population and strain on the healthcare systems. It is heterogeneous and comprised of several subgroups attributed to different cellular and molecular pathways. The most common endotype is characterized by CD4+ T helper type 2 (Th2) cells. Type 2 cytokines such as interleukin (IL)-4, IL-5 and IL-13 are released from Th2 cells and mediate airway inflammation and in most patients are suppressed by glucocorticosteroids (GCs), the front-line controller for asthma. Even with high doses of GC therapy, some asthmatics continue to experience severe symptoms. This type of asthma is associated with increases in a unique population of Th2 cells that also produce IL-17. While this novel subset of dual positive Th2/Th17 cells more strongly correlates with asthma severity than conventional Th2 cells, the molecular mechanisms driving their development are unknown. Our lab has observed that upon treatment with dexamethasone, a synthetic GC, Th2 cells exhibit higher levels of CD161 mRNA and protein. CD161 is a C-type lectin-like receptor typically associated with Th17 cells and enhancing cytokine production. With these observations, we hypothesize that GC therapy may support the expression of CD161 and the development of Th2/Th17 cells.

Methods: Primary human Th2 cells from donor peripheral blood mononuclear cells (PBMCs) will be used as the model of my study. Th2 cells are isolated based on surface expression of the prostaglandin D2 receptor, chemoattractant receptor-homologous molecule (CRTh2), reported to be the most reliable marker to identify human Th2 cells. Th2 cells are cultured on cycles of activation for 3 days and proliferation on IL-2 for 4 days. Gene expression changes will be assessed using qRT-PCR after treatment with GCs. Known markers and cytokines for Th2 and Th17 phenotypes will be assessed using surface and intracellular flow cytometry. Surface CD161 molecules on Th2 cells will be activated by cross-linking with antibody against CD161 and cytokine profile and production assessed using intracellular flow cytometry and qRT-PCR.

Results: Expression of CD161 mRNA along with an early Th17 transcription factor IRF4 increased upon treatment with GCs dexamethasone and hydrocortisone. The expression of CD161 mRNA was further increased when Th2 cells are cultured in lower IL-2 concentrations. Additionally, IL-6R, a cell surface marker essential for Th17 differentiation, was also increased in expression upon GC treatments. These findings suggest GCs may support the differentiation of the Th17 phenotype from Th2 cells.

Significance: My research may uncover that certain chronic exposures such as exogenous or stress-induced GCs may facilitate the development of a more pathogenic form of Th2, Th17 or dual positive Th2/Th17 cells which may be responsible for persistence and/or severity of asthma. Determining the driving forces of severe asthma is an essential step toward better asthma classifications and targeted therapy.

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