Public summary

Need for project

(maximum 500 characters, including spaces 497

High-grade serous ovarian cancer is the most common and lethal form of epithelial ovarian cancer. Due to the asymptonic nature at the early stage and the abdominal location of the ovary, when a patient is diagnosed as ovarian cancer, cancer cells have often already spread to the abdominal cavity. This project will address to critical needs by; 1) devising new strategies to detect micrometastases and 2) enhancing our understanding of the mechanisms that control peritoneal metastasis formation.

Goal of project (maximum 500 characters 495

Our goals are to characterize the microenvironment within the peritoneal cavity under healthy versus malignant conditions and to define how cancer cells integrate into the peritoneal lining during the formation of metastases. Our study also revealed that unique differences between the mesothelial cells that line the cavity and implanted cancer cells. We will exploit these differences to visualize very small metastases undetectable by the current imaging techniques in ovarian cancer patients.

Project description (maximum 500 characters 442

We developed highly metastatic mouse ovarian cancer models recapitulating human disease progression. Using them, we will investigate what controls different patterns of peritoneal metastasis*.* We will establish *in vitro* systems recapitulating the normal and malignant peritoneal conditions to investigate how cancer cells interact with host cells. Based on our findings, we will also develop a novel method to visualize early small metastases.

Future impact (650 649

The adjuvant therapy, debulking surgery with chemotherapy, is the current standard treatment for ovarian cancer patients. However, complete elimination of cancer cells spread to the abdominal cavity is challenging. Tiny colonies of cancer cells, which are undetectable by current imaging technologies or surgeon’s eyes, are often left behind. After this, there is a watch-and-wait period due to their invisibility. Making them visible and understanding the events during the current undetectable state are essential to fight against ovarian cancer. We believe our project will provide new insights leading to precise control of ovarian cancer metastasis.

*Scientific abstract*

*Provide a detailed summary of your research project (maximum of 4200 characters, including spaces), stating the problem to be investigated, the objectives of the investigation, the methodology to be used, as well as the significance of the research to cancer. Note that the character count may be different when copying text from Word due to formatting.*

Ovarian cancer is the fifth largest cause of cancer-related deaths in women. The vast majority of ovarian cancers are epithelial ovarian cancers and high-grade serous carcinoma (HGSC) is the most common and most lethal epithelial ovarian cancer. In the last 10 years, it has been recognized that the cell of origin of most HGSCs is within the fallopian tube epithelium, instead of the ovarian surface epithelium. However, the early events in disease progression still remain poorly defined, because HGSC is usually diagnosed at advanced stages and lack of proper animal models recapitulating human disease progression.

Using the previous CCSRI funding, we have developed a unique strategy for generating mouse ovarian cancer models, which is a combination of *in vivo* fallopian tube electroporation, Cre-mediated lineage tracing and CRISPR-mediated gene modifications. As proof-of-principle, we generated a highly metastatic HGSC model by targeting four tumor suppressor genes, *Lkb1,* *Brca1, Tp53* and *Pten.* The female mice targeted these four genes generated ovarian tumors within 5 months after electroporation and peritoneal metastasis within 6 months. After 6 months, ascites formation was observed in two third of those females. Interestingly, similar to human ovarian cancer patients, we observed two metastatic patterns, miliary and non-miliary.

Although peritoneal metastasis is the biggest challenge in ovarian cancer, its etiology and early pathophysiology are poorly understood. Our hypothesis is that **the microenvironment in the peritoneum is the important factor in ovarian cancer disease progression**. Using our unique mouse ovarian cancer model, we have three aims in this proposal;

**Aim1:** to elucidate what regulates the two peritoneal metastatic patterns, miliary and non-miliary

**Aim2:** to understand the healthy and malignant peritoneal conditions to investigate the cancer-host interaction in the peritoneum

**Aim3:** to develop new detection strategies for peritoneal micrometastases.

We believe our analysis would provide new insight into the mechanisms of peritoneal metastasis. Understanding normal and malignant peritoneal environment is essential for developing better treatment options and new drugs targeting survival of the exfoliated cells before integrating into the peritoneum. Making undetectable cancer cells visible will be highly beneficial for chemotherapy evaluation and disease control, and has a potential to use for therapeutic strategies.

*Non-confidential scientific abstract*

*For applications approved for funding, a non-confidential scientific abstract will be posted along with the funding results on the CCS research webpage.*

*Please include a duplicate of your scientific abstract – with proprietary information removed. This abstract may also be shared with potential donors and CCS stakeholders when relevant.*

*Your abstract should not exceed 4200 characters (including spaces), or roughly one full page, single spaced. Note that the character count may be different when copying text from Word due to formatting.*

*Proposal*

*Provide a scientific proposal (maximum of 25000 characters, including spaces), that includes the following: 1. the aims of the project, including any previous work done in the area. 2. study design, methods and analysis. 3. a description of the expected impact that research results will have on reducing the burden of cancer. 4. details of which member(s) of the research team will be responsible for which aspect of the project, including a rationale for their inclusion in the project and a description of the research environment where the work will take place. Note that the character count may be different when copying text from Word due to formatting. To insert special characters you must use Alt codes or the special character tool in EGrAMS and not Symbol fo*

**1. Significance (the expected impacts)**

There has been only a slight change in the mortality of ovarian cancer in the past 30 yrs. Lack of appropriate animal models, poor understanding of peritoneal environment and complex genomic alterations in ovarian cancer are part of reasons preventing understanding of early disease progression and peritoneal metastasis formation as well as devising new treatment strategies. Formation of visible and invisible innumerous metastases in the peritoneum is the major challenge of ovarian cancer. Thus, **understanding molecular and cellular mechanisms of peritoneal metastasis and establishing the experimental systems to investigate the multistep process of peritoneal metastasis formation are essential for fighting against ovarian cancer**. Furthermore, we believe that our approach for visualization of currently undetectable metastases has a potential to adopt in a clinical routine for the patients at early stages to evaluate potential peritoneal metastasis as well as for the patients received chemotherapy to evaluate the efficacy of chemotherapy and disease monitoring.

**2. Background**

*High-grade serous ovarian cancer and peritoneal metastasis*

High-grade serous ovarian carcinoma (HGSC) is the most common ovarian malignancy and is usually diagnosed at an advanced stage [2-4]. The recent recognition that this malignancy actually arises in the fallopian tube epithelium instead of the ovary has caused a paradigm shift in our understanding of HGSC. HGSCs are recognized as a cancer type strongly dominated by copy number changes with a low frequency of recurrent oncogenic mutations, few recurrent copy number alterations and high complex genomic profiles. *TP53* is the only recurrent mutation observed in all HGSCs. About half of HGSCs are associated with a deficiency in the homology directed DNA repair (HDR) pathway, with frequent genetic alterations in BRCA1/2 [4]. Recently, several copy number signatures were identified {Mcintyre, 2018;Wang, 2017}, suggesting that the multi-mutational processes in single HGSC patients contribute the complex evolution of HGSC subtypes. In addition to genomic subtyping, HGSCs show distinct peritoneal metastatic patterns; miliary and non-miliary {Eng, 2017; Auer, 2015}. The ‘miliary’ pattern shows numerous millet-like lesions spreading over the wide surface of peritoneum. On the other hand, the ‘non-miliary’ pattern shows bigger and exophytically growing implants. Although the miliary tumor spread is a strong negative factor on overall survival in human patients, little is known what regulates these two metastatic patterns. The miliary and non-miliary type metastases are not mutually exclusive can be co-present in single patients. Miliary and non-miliary are biologically different and presumably codetermined by the microenvironment in the peritoneal cavity. Interestingly, non-miliary tumors show higher EMT markers while miliary tumors show more epithelial characteristics and globally reducing metabolism {Auer, 2015}. It is tempting to speculate that miliary disease is both the source of suboptimal cytoreduction and a major predictor of poor clinical outcomes. However, little is known what regulates these two metastatic patterns.

*Peritoneal mesothelial cells, peritoneal carcinoma and ascites formation*

The peritoneum is a thin serosa membrane that lines the abdominal and pelvic cavities, envelops the intraperitoneal organs and connects anatomical compartments. Mesothelial cells form the surface lining and the source of fibroblast, smooth muscle cells and blood vessels in intestinal organs. They are quiescent under normal conditions exhibiting a flat cobblestone-like squamous epithelial morphology. Upon injury or infection, they get activated to undergo a mesothelial-to-mesenchymal transition and detach from the ECM to become free floating activated mesothelial cells. The peritoneum contains the peritoneal fluid (PF), continuously produced by mesothelial cells as a plasma transudate and reabsorbed through the large surface area of the peritoneum. The PF facilitates frictionless movement of abdominal organs, permits the exchange of nutrients, and removes pathogens and cells ascending from the female genital tract.

Ovarian cancer peritoneal spread arises from tumor cells detached from the primary tumor in the ovary/fallopian tube, consequently transported by peritoneal fluid. Although small amounts of peritoneal fluid are present in healthy women, increasing volumes of ascites are generated in many late stage patients. Ascites formation is related to a combination of altered vascular permeability and obstructed lymphatic drainage. Given the uncertainty surrounding the peritoneal metastasis process and formation of malignant ascites, the therapeutic options are limited and often the goal of treatment is to target palliation of symptoms, which can include abdominal pain, dyspnea, nausea, vomiting and anorexia. Ascites provides a local tumor microenvironment that is composed of both cellular and acellular factors, which modulate cancer cell behavior and contribute to tumor heterogeneity in ovarian cancer. The origin and phenotype of the cells in the ascites is poorly understood. Although ascites is generally considered a favorable tumor microenvironment, **our preliminary observations suggest an alternate hypothesis that ascites is a harsh environment that cancer cells barely proliferate.**

*Visualization of peritoneal metastases in ovarian cancer patients*

Peritoneal metastases (peritoneal carcinomatosis) are tightly associated with mortality of ovarian cancer [1-3]. Undetectable micrometastases spread throughout the peritoneal cavity and are missed by cytoreductive treatments (i.e. surgery and radiation therapy). They often regrow and acquire chemo-resistance in the patients.  At present, surgeons depend on visual inspection and palpation for tumor detection [4]. Current imaging methods, CT, MRI, and PET/CT have limitations and fail to detect tumor foci smaller than 1 cm. Visualizing currently undetectable micrometastases could not only help attain a complete cytoreduction of metastatic lesions [5] but also help making more precise diagnosis, prognosis and efficacy evaluation of chemotherapy. In addition, visualization of micrometastases will allow devising new strategies to eliminate or silence them.

**3. Previous work and preliminary data**

With the previously funded CCSRI innovation grant, we developed a unique strategy for generating mouse ovarian cancer models, which is a combination of *in vivo* fallopian tube electroporation, Cre-mediated lineage tracing and CRISPR-mediated gene modification (**Fig.1**). DNA/RNA/protein solutions are injected into the fallopian tube lumen, followed by electroporation with tweezer type electrodes connected to the BTX 830 electroporator. By using Cre-reporter mouse lines (e.g. *R26-loxP-stop-loxP(LSL)-RFP*), the electroporated cells are genetically RFP-labeled, permitting us to trace their fate *in vivo*. As proof-of-principle, we tested whether we could generate a novel HGSC mouse model. We selected four genes linked to HGSC tumorigenesis, *Trp53, Brca1, Pten* and *Lkb1* [12,13]. *TP53* is mutated in all HGSC [1]. *BRCA1,* as well as *BRCA2,* is deficient in about half of HGSC and is a well-known familial cancer gene linked to HGSC, involved in the homology directed DNA repair pathway. *PTEN* is also frequently downregulated or deleted in HGSC [6,16], while downregulation of *LKB1* was reported in 70% of cases [17] and its locus (19p13.3) is frequently deleted [16]. We generated sgRNAs against*, Trp53, Brca1* and *Pten* and subcloned them into a PX330 plasmid that enables co-expression of sgRNA and Cas9 in mammalian cells. These PX330 plasmids along with a Cre-expressing plasmid were electroporated into the fallopian tube luminal epithelium of 4-6 week old females (*R26-LSL-RFP* or *Lkb1fl/fl;R26-LSL-RFP*). Using this method, mutations in the targeted genes were introduced by CRISPR/Cas9 and, simultaneously, *Lkb1* deletion and *RFP* activation were induced by the Cre recombinase (**Fig.2**). To confirm that the desired mutations were properly introduced, we isolated RFP+ and RFP- cells at 1 month after electroporation and performed targeted sequencing on PCR-amplified regions. We found that only RFP+ cells had mutations in *Brca1*, *Trp53* and *Pten* while no mutations were found in the RFP- cells.

At 4 months after electroporation, only in the *Lkb1* deletion cohort, serous papillary tumors were observed on the ovarian surface (5 out of 6 ovaries from 3 mice) (**Fig.3A,D**). Remarkably, in as little as 6 months after electroporation, peritoneal metastasis formed in the *Lkb1* deletion cohort (**Fig.3A,E** 19 out of 20 mice) and many mice generated abdominal ascites (**Fig.3B** 15 out of 20 mice). Interestingly, **we also identified two patterns of peritoneal metastasis; ‘miliary’ (4/16 cases) and ‘non-miliary’ (12/16 cases) similar to human patients** (**Fig**.**3C).** Most our ovarian tumors exhibited the high-grade serous phenotype (**Fig.3F,G,H**) but some mice also had high-grade endometrioid and high-grade carcinosarcoma (carcinoma+sarcoma) phenotypes (**Fig.3G**) with characteristics of high-grade malignant mixed mullerian tumors (MMMTs). Analyses of mutation patterns of targeted genes are currently ongoing using MiSeq sequencing of targeted loci of ovarian tumors and metastatic lesions. **Preliminary MiSeq analysis revealed allelic frequency in primary ovarian tumors and metastatic lesions in single females and allowed us to predict clonal selection during cancer progression** (**Fig.4**). In the left ovarian tumor, clone2 and 3 contributed ovarian tumors but did not progress to peritoneal metastases. We are currently investigating the relationships between genotypes of targeted loci, cancer histotypes and metastatic patterns.

Because cancer cells were labeled with RFP, we could identify early micrometastases on the visceral peritoneum without obvious floating tumor cells/spheroids and ascites formation (**Fig.5A**). They were integrated to the mesothelial layer but not invading underneath. Some relatively large micrometstases (approx. 500µm diameter) showed the corral-like morphology (**Fig.5B**) while smaller ones (~200µm diameter) showed tight compact colonies clearly distinct from the surrounding flat mesothelial cells (**Fig.5C**). This surface-spreading pattern of metastases was also observed in the females at the later stages with ascites (**Fig.6**). The metastases grew on the peritoneal surface forming fragile papillary tumors, similar to the disease condition of some patients. The result clearly indicates that early metastasis formation is independent of ascites formation.

Two third of the *Lkb1* deletion cohort formed ascites 6 months after surgery. We performed preliminary 10x Genomics single cell transcriptome analysis on the floating ascites cells from a single female (**Fig.7**). The majority of cells in the ascites were host immune cells, particularly, macrophages and dendritic cells. Granulocytes and neutrophils were also present. T-cells (*Cd3g*, *Zap70*) were a quite minor population in the ascites. Activated floating mesothelial cells expressed many markers for cancer associated fibroblasts (CAFs) supporting the idea that they are one of sources for CAFs {REF}. Interestingly, epithelial cancer cells *(Cldn4, Pax8, Wt1, Cdh1* positive cells) were a minor population in the ascites. Since we also observed RFP positive floating cancer spheroids, we speculate that most of epithelial cancer cells were not present as single cells but in the spheroids. Unknown mesenchymal cell population was also present (*Wt1, Cdh2, Vim, Osr1* positive, *Pax8, Cdh1* negative).We are currently investigating if *Rfp* expresses in this population. At this point, it is unclear if they are cancer cells however, if yes, we will examine how they clonally related with the epithelial cancer cells.

Although ascites is generally considered as a favourable condition for cancer cells, we found that ascites was acidic (approx. pH5) and hypoxic, indicated by *Hif1a* expression in 10x genomics single cell transcriptome analysis (**Fig.6**). The preliminary semi-quantitative metabolomic analysis (**Fig.8D**) showed taurine, which is involved in osmoregulation, was reduced to only 4% in the ascites compared to normal PFs. On the other hand, 2-OH-3-MeButylic acid and Glucose were 50 and 10 times higher in the ascites, suggesting lactic- and keto-acidosis. In addition, the floating cancer spheroids were Ki67 negative (**Fig.8C**). Interestingly, the surface of some papillary tumors was covered with host cells with high YAP and b-catenin staining (likely activated mesothelial cells) (**Fig.8A,B**). We speculate that, in order to form large tumor nodules, cancer cells need to be integrated into the sub-mosothelial zone to escape from the harsh acidic environment and to attract blood vessels. On the other hand, cancer cells directly contacting the ascites fluids forms corral-like papillary tumors or spheroids, which are highly fragile but could survive in the harsh condition. Since they are not highly proliferative populations, we speculate that they could be the population surviving through chemotherapy. Therefore, it is important to understand the malignant peritoneal condition and how cancer cells behave and interact with host cells in this harsh environment, which is a sharp contrast to the current *in vitro* culture conditions for drug screening.

Taken together, our unique strategy has several advantages over the current mouse cancer models; 1) Saving time and costs to generate mouse models because no breeding is required to generate cohorts; 2) High flexibility permitting many gene combinations/modifications and host genetic backgrounds to be tested; 3) control over the size and area of targeted cells (the low-frequency mosaic transfection pattern better recapitulates the sporadic nature of human tumorigenesis); 4) The ability to track genetically modified cells by fluorescent reporters, permitting analysis of tumor initiation and early metastasis; 5) The ability to track clonal evolution by sequencing; 6) Highly metastatic mouse models with immune competency.

**4. Hypothesis and the aims of the project**

Our hypothesis is that **the microenvironment in the peritoneum is the important factor in ovarian cancer disease progression**. To address this hypothesis, we have three aims;

**Aim1:** To elucidate the contribution of the microenvironment and tumor cell genotypes in the formation of two metastatic patterns; miliary and non-miliary.

**Aim2:** To understand healthy and malignant peritoneal microenvironment and establishing the *in vitro* system to investigate the interactions between exfoliated cancer cells and host mesothelial cells.

**Aim3:** To visualize peritoneal micrometastases in ovarian cancer patients.

A schematic view of this proposal is in **Fig.11.**

**5. Experimental Design, Methods and Analysis**

*Aim1: To elucidate the contribution of the microenvironment and tumor cell genotypes in the formation of two metastatic patterns; miliary and non-miliary.*

Miliary and non-miliary metastases are not mutually exclusive and can be observed in a single patient. At this point, it is unknown what causes the two distinct metastatic patterns. Two possibilities are considered; clonal selection and contribution of microenvironment. In our preliminary study, we also observed the two metastatic patterns in a single animal. As we are currently analyzing clonal selection using MiSeq data (**Fig.5**), the peritoneal metastases in the preliminary study were likely multiclonal origins due to bilateral electroporation and high electroporation efficiency (1- 5 clones/ fallopian tube). To recapitulate human disease progression originated from a single clone, we will perform unilateral fallopian tube electroporation with a limited number of electroporated cells by reducing the number of electroporation pulses. To determine if generated tumors are mono- or multiclonal, we will use ROSA-LSL-Confetti mice (**Fig.1**)[26]. In this Cre-reporter line, Cre activation randomly marks individual cells with one of four different colors. Therefore, if tumors show different colors, it indicates they are emerged from multiple electroporated cells, while if they show a single color, they are likely emerged from a single electroporated cell. We will confirm this further with genome sequencing.

Using the females carrying monoclonal ovarian cancer, we will investigate if the miliary and non-miliary patterns are co-emerged in single mice by 5-8 months after surgery. 10x genomics single cell analysis on primary ovarian tumor and metastatic lesions will be performed to identify the cell populations consisting primary and metastatic tumors (6 females including miliary, non-miliary and mixed metastatic patterns, (1 ovarian tumor + 4 lesions)/female). Using the residual cells of the same mice (if enough) or tumors from another females, we will separate cancer and host cells and perform deeper transcriptome RNAseq analysis with pooled RNAs and whole exome sequencing (WES) analysis to identify the mutation patterns of the targeted four genes as well as other possible deletions. WES would be useful to identify if any unique deletion pattern is selected beside the targeted four genes. Based on these sequencing analyses, we will identify unique cell population and gene signature in cancer and host cells as well as mutation patterns in cancer cells. To investigate the distribution of specific cell types in the 3D space, we will perform 3D confocal and light sheet microscopic analyses on thick vibratome sections (50-100µm) and Opal multiplex 7-color immuno fluorescence on traditional tissue sections. These analyses will reveal if the miliary and non-miliary differences are caused by clonal selection of cancer cells or plastic phenotypes regulated by the interaction with specific host cells. In addition, we will investigate if miliary and non-miliary are fixed phenotypic traits by tumor transplantation experiments to nude or isogenic B6 females. Dissociated tumor cells isolated from miliary or non-miliary peritoneal lesions will be injected into the peritoneum cavity. The frequency and types of metastasis will be analyzed after 2-4 month of injection.

Because our mouse ovarian cancer models will be originated from single clones, we will be able to track clonal evolution and its link to metastatic patterns and disease progression, similar to the recent genomic study on mouse pancreatic cancer models {Mueller, 2018}. If we observe unique deletion patterns, we will perform array comparative genome hybridization (aCGH) or whole genome sequencing (WGS) to capture genomic changes and clonal selections in single mice.

*Aim2: To understand healthy and malignant ascitic peritoneal microenvironment and devise an in vitro system to investigate the growth of micrometastases and the interactions of cancer and mesothelial cells.*

To form peritoneal metastases, the exfoliated cancer cells must survive in the peritoneal environment; adhere to the surface of the peritoneum and grow out as papillary tumors (**Fig**.**7** many of miliary metastases) or integrate into the sub-mesothelial space (non-miliary metastases). Various molecular events must cooperate for cancer cells to efficiently attach and adhere to the peritoneal lining, but limited information is available. Development of novel *in vitro* experimental models, recapitulating *in vivo* healthy and malignant conditions is needed. It is worth to note that the current tissue culture conditions (e.g. adhesive dishes, media compositions, pH, CO2) are historically established for supporting highly selected cancer cell lines to proliferate rather than to recapitulate *in vivo* conditions.

Mesothelial cells *in vivo* are quiescent but become highly proliferative *in vitro* as mesenchymal fibroblasts {REF}. However, in our knowledge, there is no method to restore *in vivo* mesothelial cell property in these cultured mesothelial cells. Therefore, little is known about their normal quiescent property. To investigate their normal characteristics and interaction with cancer cells,we developed a unique tissue preparation method to isolate the serosa membranes, like mesentery and omentum, which usually are lost during isolation without a supporting structure. Using a pair of two-sized plastic O-rings, we punch out the mesentery/omentum to maintain the isolated material as a flat tissue with membranous morphology (**Fig.9A-C**). This preparation method allows us to observe the tissues under a microscope and culture them *in vitro* (**Fig.10**). In the mesentery explant from healthy mice (no tumors), the mesothelial cells were highly quiescent. The most cells have no F-actin structure with phalloidin staining, thus cellular outlines were hardly visible. In small areas, some flat F-actin positive cells were occasionally observed, might be showing homeostatic turnover or local activation. Consistent with this, the mesentery explants isolated from a Lifeact-GFP female were GFP negative at the time of isolation. Interestingly, GFP was turned on within 24 hrs in several different culture conditions (**Fig.10**), suggesting that the *in vitro* culture with standard culture media activated mesothelial cells. To develop better culture conditions similar to the peritoneal environment that keeps the cells at the quiescent stage, we will perform the quantitative metabolomic analysis to identify the metabolite composition and concentration in the normal and malignant peritoneal fluids (3 healthy PF + 3 ascites). We will also use peritoneal dialysis solutions (e.g. Dianeal, Baxter pH5) under a hypoxic condition supplemented with amino acids based on our metabolomic analysis. For evaluation of culture conditions, we will use the mesentery explants isolated from Lifeact-GFP females. We will also examine the supernatant of mouse malignant ascites if they activate mesothelial cells or not. Once we identify the proper culture media conditions, we will place our mouse primary ovarian cancer cells and/or human ovarian cancer cell lines, like OV90 and OVCAR3{Habyan,2018; Klymenco,2017} on the surface of the explants to investigate how cancer cells integrate to the mesothelial layer. We will also isolate the mesothelial explants with micrometastases from our ovarian cancer model and culture them in the ascites fluids or our newly identified culture conditions. We will perform live-imaging analysis on these explants (e.g. Lifact-GFP explants with RFP cancer cells) to investigate how cancer cells integrate and spread on the surface of the peritoneum, whether EMT is required and how the corral-like papillary morphology grows.

*Aim3: To visualize peritoneal micrometastases in ovarian cancer patients.*

We have noticed that peritoneal micrometastases were forming morphologically unique colonies on the peritoneum (**Fig.5**).   By staining with F-actin, micrometastases were clearly distinct from surrounding peritoneal mesothelial cells that are in quiescent and negative of F-actin (**Fig.5B,C**). Although identification of these small colonies is very difficult by traditional pathological tissue sectioning (i.e. making tissue blocks and sectioning), they are clearly identifiable when the tissues are observed from their surface. We hypothesize that the peritoneal micrometastases undetectable using current clinical imaging and hard-to-find in pathological sectioning can be visualized by a combination of simple fluorescent F-actin staining with surface inspection by a fluorescent microscope/laparoscope in human ovarian cancer patients.

To test this, we will collect fixed peritoneal tissue samples from the patients receiving a debulking surgery and stain with dyes (Alexa488-phalloidin or Sir-Act) that can visualize F-actin.   We will collect several strips of patients’ peritoneum tissues like omentum and mesentery from 10 ovarian cancer patients with peritoneal mestastases. The strips will be fixed in 4% PFA solution and stained with Alexa-phalloidin. Then, they will be analyzed under a dissecting fluorescent microscope or a laser confocal microscope.

To explore if this strategy can be used inside of patients in the future, we will investigate whether SiR-Act, a live cell F-actin probe, can be used in the peritoneum of live animals. The treatment of verapamil with SiR-Act is recommended to enhance the signal during uptake of the probe. We will optimize the SiR-Act treatment conditions in our mouse ovarian cancer models.

**6. Research Team and Environment**

Dr. Yojiro **Yamanaka (lead PI, GCRC McGill)**

Dr. Anne-Marie **Mes-Masson** (**co-applicant**, CHUM,Director of FRSQ Cancer Research Network)

Dr. Diane **Provencher** (**co-applicant**, CHUM, Gyneco-oncologist)

Most of proposed experiments will be performed in the Yamanaka lab at GCRC, McGill. Human tissue sample collection and their primary processing will be performed at CHUM.

Dr. Ioannis **Rougousis** (Head of Genome Sciences, McGill University and Génome Québec Innovation Centre) will support 10x Genomics single cell analyses. For sequencing analyses, we are receiving bioinformatic support from Drs Ken **Dewar** (McGill, Génome Québec Innovation Centre) for MiSeq analysis and Alain **Pacis** (GCRC Bioinformatician) for WES/WGS analysis. Dr. Jocelyne **Arseneau** (Director of Gynecological Pathology, MUHC) will provide her expertise for pathological evaluation. Dr. Daina **Avizonis** (Director of GCRC metabolomics core)

(25503)

*Innovation to Impact statement (applicable only to currently funded Innovation Grant holders)*

*Briefly describe (maximum of 2100 characters, including spaces) the achievements resulting from the original Innovation Grant and how this proposal will build on these outcomes. Note that the character count may be different when copying text from*

*Word due to formatting.*

1587

Using the previously funded CCSRI innovation grant, we developed unique mouse ovarian cancer models, which are highly flexible in mutation combinations and targeting area, highly metastatic with immune competency and the ability to track the exfoliated cancer cells in the peritoneum. In our knowledge, our models are the only models to recapitulate the two peritoneal metastatic patterns; miliary and non-miliary, similar to the human patients. Based on the observation we made in our models, we will investigate the peritoneal environment and its interaction with the foliated cancer cells. We believe our analysis would provide new insight into their metastatic mechanisms. In addition, our mesentery explant culture to study mesothelial cells is a unique innovative experimental method. Importance of the peritoneum has been recognized in abdominal surgery and peritoneal metastasis of various cancers. However, molecular and cellular understanding of the peritoneal mesothelial cells has been limited due to lack of appropriate experimental methods. Understanding normal and malignant peritoneal environment is essential to understand how the exfoliated cells survive and integrate into the peritoneum. Lastly, based on the preliminary observation in mice, we will explore the possibility to devise a new strategy to visualize currently undetectable micrometastases in the peritoneum of ovarian cancer patients. Making undetectable cancer cells visible will be highly beneficial for chemotherapy evaluation and disease control, and has a potential to use for therapeutic strategies.

*Vision statement*

*Describe how the proposed work will move the field forward and accelerate progress in cancer research (maximum of 1000 characters, including spaces). This statement should also clearly address the expected “next steps” following completion of the project. Note that the character count may be different when copying text from Word due to formatting.*

*916*

There has been only a slight change in the mortality of ovarian cancer in the past 30 yrs. Lack of appropriate animal models, poor understanding of peritoneal environment and complex genomic alterations in ovarian cancer are part of reasons blocking our understanding of early disease progression and peritoneal metastasis formation. Invisibility of cancer cells is the biggest challenge in the field to understand the emergence of malignant cancer and how they spread and grow as metastasis. We will make the current undetectable cancer cells visible, first in out models, second in patient’s pathological specimens and eventually in patient’s peritoneum. Making undetectable cancer cells visible helps for early detection, precise disease monitoring and efficacy evaluation of chemotherapy. I have an ambition to combine our visualization strategy with photochemo- or photoimmune therapy to eliminate peritoneal micrometastases.

25.

Tables, graphs, charts and associated legends

OPTIONAL: Attach and appropriately label figures, graphs, charts and legends in PDF format (maximum of 5 pages total).

NOTE: For the file name, please use the following format: [lastname\_firstname-figures]