Histology, Whole Slide Scanning, & Image Analysis Core Service at Stephenson Cancer Center

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https://www.ouhsc.edu/pathologyJTY/download/SCC-Histology-Core.PDF

Stephenson CANCER CENTER

Overview

Services are provided by the Tissue Pathology Shared Resource and Histology, Immunohistochemistry, & Microscopy Core at the Stephenson Cancer Center. The mission of these two cores are to facilitate and promote the study of cancer using clinical & experimental specimens, & to enhance quality, efficiency, & productivity by providing technical expertise & services. The two cores are led by a board-certified pathologist. Services include:

1. Consultation on experimental design & interpretation.
2. Histologic processing, sectioning (paraffin & frozen section), and a variety of conventional stains including hematoxylin-eosin, Masson trichrome, Sirius red, Luxol fast blue, and other stain. These cores also provide automated immunohistochemistry, immunofluorescence, in situ hybridization (chromogen or fluorescence based) using products from Advanced Cell Diagnostics, and TUNEL.
3. Construction of tissue/cultured cell microarray.
4. Bright-field & fluorescence whole slide scanning, digital photomicrography (bright-field, dark-field, polarized light, & fluorescence).
5. Whole slide scanning based image analysis, bright-field & fluorescence.
6. Facilitates the use of archival human pathology sample from the Department of Pathology.

Staffing & Equipment

- Four full time and three part-time staffs including the director who is a board certified anatomic pathologist.

Equipment

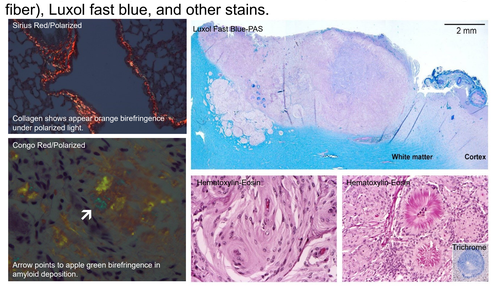
- Histology: Tissue Processor (Leica TP1020), Embedding Center (Leica EC1150), Cryostat (Leica CM1950), Microtome (Leica RM2255), Cassette labeler (Brady BSP31), Label Maker (Brady BBP11), Cytospin (Thermofisher Cytospin 4), Tissue Arrayer (Veridiam).
- Staining: Conventional Stainer (Leica ST 5020); Immuno-histocheistry, *in situ* hybridization, TUNEL (Leica BOND III & BOND RX).
- Photomicrography: Bright-field, fluorescence, dark-field, polarized.
- Whole slide scanner (bright-field & Fluorescence): Leica-Aperio CS, Zeiss AxioScan.Z1.
- Image analysis: Leica-Aperio Tool Box, TMA segregation, in situ hybridization quantifier; Indica HALO Plus 10 (undergoing installation).

Consultation & Interpretation of Results

- Our director, a board certified (American Board of Pathology) pathologist with extensive experience working with human and experimental tumors, provides assistance on experimental design and interpretation of stained sections. The interpretation can be a major help when human tumor samples are used in the study.

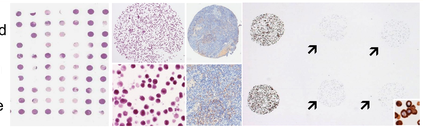
Histology Service & Conventional Staining

- **Services available:** We provide fixation, tissue processing and embedding, sectioning of paraffin blocks and frozen blocks, decalcification, cryoprotection, paraffin curls cutting.
- **Conventional Staining:** We provide a full line of conventional staining including hematoxylin-eosin, Masson trichrome, Sirius red (for collagen fiber), Luxol fast blue, and other stains.



Tissue/Cultured Cell Microarray

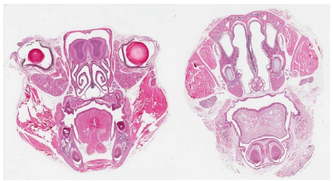
- Tissue microarray (TMA) and cultured cell microarray (CCMA) can be constructed from paraffin blocks and used for IHC, ISH, IHC+ISH, and other staining techniques.
- TMA is a cost effective way to study expression of certain molecules in a high number of specimens. When using a 0.6 mm core, hundreds of samples can be packed on one paraffin block.
- CCMA can be used to screen multiple cell lines quickly. This avoid the cost and time of growing up individual cell lines every time.



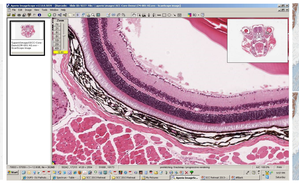
TMA, HE stain. CMA, HE stain. TMA-IHC for NFP. CMA, IHC for cytokeratin 7. Negative cells are highlighted by arrows.

Whole Slide Scanning & Image Analysis

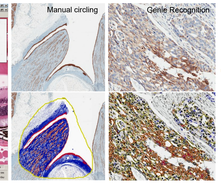
- **Scanning:** Bright-field scanning of the entire histologic section, cytologic preparation, and TMA/CMA up to 40x are provided by an Aperio scanning system. Both bright-field & fluorescence whole slide scanning up to 40x at a NA of 0.95 is provided by the Zeiss AxioScan.Z1 scanner.
- **Image analysis:** Aperio system provides a variety of image analysis with and without Genie morphologic recognition. These quantitative analysis includes pixel count, membranous count, cytoplasmic count, nuclear count, blood vessel density count, and others. TMA segmentation is available. Files can be copied out for further analysis using a third party software. HALO Plus 10 Image analysis software (3 site licenses) are undergoing installation and will be available soon with two of the licenses allowing remote access.
- **Remote assess:** Aperio allows remote and simultaneous assess by multiple users which foster discussion and exchange of ideas. Remote access for the Zeiss AxioScan.Z1 is undergoing installation and will be available soon.



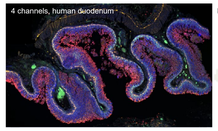
Whole slide image of a murine head at coronal plan. Whole slide image is an excellent way to generate panoramic view of large slides that cannot be generated by traditional microphotography.

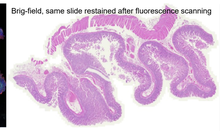


Whole slide image of a murine head at coronal plan and details of the retina at 40x.

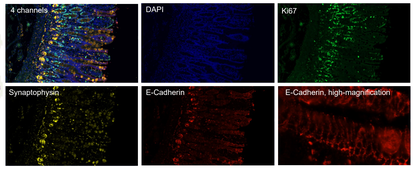


Quantitative analysis using manual circling and Genie recognition on IHC.



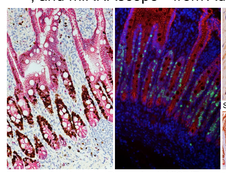


Re-staining Double Scanning (RSDS): Fluorescence slide is first scanned, the cover slip is then taken off, stained with a conventional bright-field stain such as hematoxylin-eosin or trichrome, and the slide is rescanned.

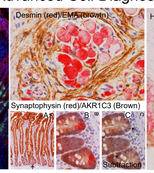


Immunofluorescence, Immunohistochemistry, & *in situ* Hybridization

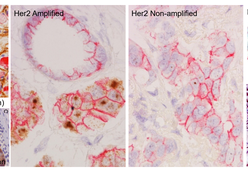
- **Immunohistochemistry (IHC)/Immunofluorescence (IF):** Both are automated and works with most antibodies.
- **In situ hybridization (ISH):** Chromogen or fluorescence probe based. Automation is coupled to RNAscope®, BaseScope®, and miRNAscope® from Advanced Cell Diagnostics®. Can combine with immunohistochemistry & immunofluorescence.



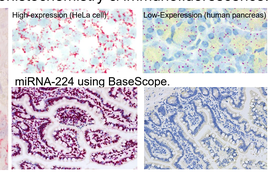
Human duodenum. Brown/red: Ki67/E-cadherin. Green/red: Ki67/E-cadherin



Double IHC with & without subtraction.



Combined IHC & ISH. Brown: ISH for Her2 (RNAscope), Red: E-cadherin.



miRNAscope. Human duodenum. Left: control positive. Right: control negative.

Usage of Human Pathology Specimens

TPSR coordinates (with IRB approval) with the Department of Pathology in retrieval of archival formalin fixed paraffin embedded human tumor samples and also access to clinically used immunohistochemistry in the hospital.

Support

These two cores are supported by grant NIH/NCI 1 P30 CA225520-01, NIH/NIGMS 2 P20 GM103639, NIH/NIGMS 1 S10 OD026744, multiple equipment grants from the Presbyterian Health Foundation (PHF), the Department of Pathology and Stephenson Cancer Center of the University of Oklahoma Health Sciences Center.

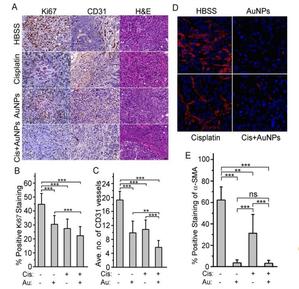
Impact

High-quality histology, whole slide scanning, & image analysis need substantial investment, maintenance, & skilled staff. These cores provide services in a cost effective way that allow scientists to cross this barrier. In addition, these cores facilitates team research between cell biology, experimental oncology, pharmacology, pathologists and others working together.

Scientific Highlights

Ovarian Cancer and Gold Nanoparticles: Dr. Priyabrata **Mukherjee (PTCR)** discovered the unique anti-angiogenic property of gold nanoparticles (GNPs) that inhibited tumor growth and metastasis, and sensitized ovarian cancer cells to cisplatin therapy by reversing epithelial-mesenchymal transition (EMT), inhibiting MAP-Kinase activation and depleting cancer stem cell (CSC)-like cells. The TP SR coordinated development of a 125 patient ovarian cancer tumor microarray (TMA) in collaboration with Dr. **Zuna (GC pathologist)** and Dr. **Mukherjee**. In addition, TP SR provided histology, IHC, and image analysis of the xenograft models with an ovarian cancer cell line treated with gold nanoparticle with and without cisplatin.

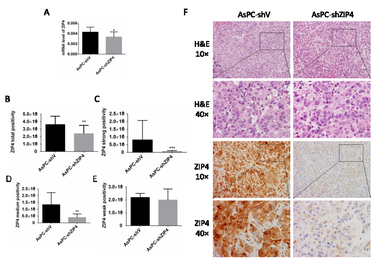
Outcome: This project led to the renewal of an R01 application (CA136494; PI, **Mukherjee**). Related publications include: *Oncotarget* 2014, 5(15):6453; *Oncotarget* 2015, 6(35):37367.
Critical support from TP SR : IHC, TMA (human ovarian tumor archival materials, Image Analysis
SCC Program: PTCR, GC



Treatment with and without gold nanoparticles: (A) Representative histology of tumors from mice xenografts of SKOV3-ip cells with Ki67 and CD31 expression. (B) Image analysis of Ki67 staining (C) Image analysis of CD31 staining analysis. (D) Immuno-histochemistry / immuno-fluorescence staining of mice tumor tissues with α-SMA antibody. (E) Image analysis of α-SMA staining.

Pancreatic Cancer and ZIP4 Silencing: Dr. Min **Li (PTCR)** focuses on the role or ZIP4 and its effects on downstream signaling in murine pancreatic carcinoma xenografts with and without ZIP4 expression by silencing ZIP4 expression with small RNA. Quantitative image analysis stratified the staining intensity into three tiers and demonstrated that low level of expression was not affected by the silencing. However, medium and especially high level of expression of ZIP4 was strongly attenuated by the small RNA. These results could not be obtained by manual evaluation without digitized image analysis.

Outcome: This project led to one NCI R01 grant (CA203108; PI, **Li**) focusing on ZIP4-mediated pancreatic cancer cachexia.
Critical support from TP SR: IHC, image analysis, interpretation of results, histology. **SCC Program:** PTCR

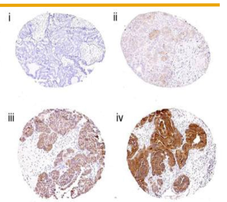


Silencing ZIP4 with shRNA suppresses expression of ZIP4 in murine pancreatic carcinoma xenografts. (A) mRNA level of ZIP4 in orthotopic pancreatic cancer xenograft tissue in AsPC-shV and AsPC-shZIP4 groups. (B-E) Positivity analysis of ZIP4 staining in orthotopic pancreatic cancer xenograft. Weak, medium, strong positivity were calculated through the positive intensity relative to total area of xenograft tumor. (F) H&E and ZIP4 staining in orthotopic pancreatic cancer xenograft.

Treatment Resistance Ovarian Tumor: Dr. Sukyung **Woo's (GC)** research focuses on mechanisms of therapeutic response and resistance to anticancer agents targeting tumor microenvironment. Her laboratory developed phenotypically and acquired xenograft tumor models of ovarian cancer resistant to antiangiogenic therapeutics to identify potential alternative pathways, such as CXCR2 and apelin, targeting ovarian tumor microenvironment. The TP SR provided histology, IHC, and quantitative image analysis of microvessel density, cell proliferation, hypoxia, CXCR2, and apelin and its receptor APJ. High level expression of APJ was strongly associated with reduced overall survival of patients with high grade serous carcinoma. This work has been published in *PLoS One* 2015, 10(9):e0139237.

Outcome: This project led to an ACS Research Scholar Grant (RSG-16-006-01-CCE, PI, **Woo**) funded in 2016, a pre-proposal for the DoD Ovarian Cancer Research Program (which has been invited for full proposal submission), and a new NIH R01 in preparation that will be submitted later this year.
Critical support from TP SR : Histology, IHC, image analysis. **SCC Program:** GC

Ovarian Cancer and Treatment Resistance: Dr. Resham **Bhattacharya (GC)** focuses on the role of BMI1, a stem-cell self-renewal protein, in regulating high-grade serous ovarian carcinoma progression and chemoresistance. Initial data demonstrated loss of BMI1 potentiates apoptosis via the DNA damage response pathway. Poor survival is associated with overexpression of BMI1 secondary to under expression of microRNA 15a/16. Based on these studies Dr. **Bhattacharya** was awarded an NCI R01 (CA157481) to investigate how BMI1 mediates resistance to cisplatin in high-grade serous ovarian carcinoma. She also focuses on the biological significance of TGFβ in uterine carcinosarcoma, an uncommon malignancy with both epithelial and mesenchymal differentiation.



Expression of BMI1 in ovarian tumor microarray. Immunohistochemical staining of a tissue microarray of epithelial ovarian cancer samples. Representative images are shown of none (i), weak (ii), moderate (iii), and (iv) strong staining.

Her laboratory demonstrated up regulation of components of the TGFβ pathway in recurrent tumor versus the non-recurrent tumors and that Galunisertib (LY2157299, Eli Lilly) efficiently attenuated the proliferation, migration, and epithelial-to-mesenchymal transition (EMT) likely responsible for the invasive and aggressive nature of UCS and resistance to therapy.
The TP SR has been instrumental in providing archival human tumor paraffin blocks for the construction of a TMA in order to attain statistically meaningful results for these human tumors on drug discovery.

Outcome: A multi-PI R01 has been submitted to the NCI that will evaluate efficacy of Galunisertib along with standard carboplatin/paclitaxel chemotherapy in pre-clinical models of uterine carcinosarcoma (*Oncotarget* 2015, 6:14646). Additionally, a phase 1b feasibility IIT of Galunisertib will be initiated later in 2016 at the SCC (PI, Kathleen **Moore**; translational co-PI, **Bhattacharya).**
Critical support from TP SR: Providing human tumor archival material TMA, histology, IHC. **SCC Program:** GC

Selected Publications

- Chakraborty PK, Mustafi SB, Xiong X, Dwivedi SKD, Nesin V, Saha S, Zhang M, **Dhanasekaran D (GC),** Jayaraman M, **Mannel R (GC), Moore K (GC), McMeekin S (GC), Zuna R (GC),** Ding K, **Tsiokas L (PTCR), Bhattacharya R (GC)** and **Mukherjee P (PTCR).** MICU1 drives glycolysis and chemoresistance in ovarian cancer. *Nat Commun.* 2017, 8:14634.
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- Saha S, Chakraborty PK, Xiong X, Dwivedi SK, Mustafi SB, Leigh NR, Ramchandran R, **Mukherjee P (PTCR)** and **Bhattacharya R (GC)**. Cystathionine β-synthase regulates endothelial function via protein S-sulfhydration. *FASEB J.* 2016, 30:441-56.
- De Souza PC, Balasubramanian K, Njoku C, Smith N, Gillespie DL, Schwager A, Abdullah O, Ritchey JW, **Fung KM (NA),** Saunders D, Jensen RL and **Towner RA (PTCR).** OKN-007 decreases tumor necrosis and tumor cell proliferation and increases apoptosis in a preclinical F98 rat glioma model. *J Magn Reson Imaging.* 2015, 42:1582-91.
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