Innovations

Using Cancer Staging to Teach about Tissue Layers

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Abstract: Histological assessment of tissues is a critical component of diagnosing diseases and determining disease severity or stage. In order to fully appreciate tissue pathology, an understanding of healthy tissue layers is required. Students traditionally learn this information by memorizing different layers within healthy tissue. This is often a daunting task if not placed into a meaningful real-world context. Described here is a hands-on inquiry-based laboratory where students make a diagnosis based on their knowledge of tissue layers. Students are provided readily available preserved tissue from two patients. Students are unaware that one patient is normal while the other has bladder cancer. Students stain the tissues using a common histological technique and are responsible for providing a bladder cancer diagnosis with corresponding cancer stage. This activity not only provides real-world technical experience, but since cancer staging is determined by the tissue layers the tumor has infiltrated, it also reinforces the importance of knowing different tissue layers. Following their diagnosis, pathology reports are assembled using images from either a microscope or cell phone. Students then compare these results with professional pathology reports accompanying the tissue. This activity is well suited for health sciences, cancer biology, histology, or pathology classes.

Key words: Histology, cancer, pathology

Introduction

Teaching students about basic histological features of tissues can be a challenging task. This is often accomplished by asking students to observe and memorize various layers using prepared slides of healthy tissue. However, many students have trouble retaining this information or appreciating its value when not connected to a medical application. Conversely, students are much more engaged in learning about tissue layers after their importance in disease diagnosis becomes clear. Described is an inquiry-based learning activity where students identify different tissue layers and compare normal vs. pathological cancer tissues. While cancer could be explored in various organs, bladder cancer is an ideal tissue type for this activity. A bladder cancer diagnosis requires the ability to recognize abnormal tissue and the severity/stage of the cancer is determined by identifying which tissue layers the tumor has infiltrated, as described by commonly used cancer staging guidelines (the Tumor-Node-Metastasis (TNM) Staging System). Bladder cancer is also the 9th most commonly diagnosed cancer worldwide and the 13th most common cause of death (Antoni et al., 2016), thus highlighting the importance of its diagnosis. Importantly, stained and unstained bladder cancer tissue is readily available for purchase at various tissue repositories.

The goal of this inquiry-based activity is for students to assess and diagnose bladder tissue samples. To do this, students are provided tissue from two separate patients experiencing various symptoms. While the symptoms for both patients are provided to students, diagnoses are not provided. Students then stain these tissues using hematoxylin and eosin (H&E) to visualize tissue composition. Using their knowledge of normal bladder tissue, students determine which tissue is diseased and what disease that patient has. This activity typically requires two lab periods. The first lab period is devoted to completing the H&E stain. In the second lab period, students visualize and assess their stained tissues. Students also acquire images of their tissues by placing a cell phone to the oculars of the microscope or by using a dedicated microscopy camera. These images are then used to assemble a pathology report (see report outline below).

Methods and Materials

Lab Period 1
Activity Introduction: To prepare students for this activity, students were provided background information on the two patients. This included their age, gender, and symptoms. Symptoms were generated by the instructor based on known bladder cancer symptoms and were identical for both patients (see pathology report section below for a detailed list of symptoms). Students were then briefly introduced to de-identified patient IDs, tissue procurement, paraffin embedding, and H&E staining.

Bladder Tissues: Paraffin-embedded human bladder tissue sections were obtained from The UConn Health Research Biorepository. H&E stained and unstained tissue sections from non-cancerous normal individuals and individuals at various cancer stages can be purchased from the Biorepository for approximately $7 per slide. Tissue sections are sent pre-mounted to individual glass slides, which can be stored long-term and subsequently stained when needed. To observe a range of disease stages and patient diversity, each student group can be provided tissue from different bladder cancer patients. By doing this, early (T1), intermediate (T2a, T2b), and late-stage (T3b) cancer can be observed. Moreover, each patient shows unique pathological characteristics, including variability in tumor size, tissue layer infiltration (i.e., stage), fibrosis, and inflammation. As described below, student groups can briefly share images of their tissue with the entire class so all students gain an appreciation for disease stages and diversity.

Description of Patient Samples: In compliance with HIPAA regulations, patient samples provided by the Biorepository have been de-identified. Instead of investigating a patient sample based on their name (e.g., John Smith), students investigated patient samples using unique patient ID numbers (e.g., patient 08-29). Information regarding the gender and ages of the patients is provided.

Hematoxylin and Eosin Staining: Mounted tissue slides from one cancer patient and one normal control patient (labeled with their patient ID numbers) were provided to each group of 3 students and placed in glass coplin jars in preparation for staining. Tissue sections were deparaffinized with two washes of xylene (2 mins each). The tissue was then rehydrated using sequential ethanol washes. To do this, tissue was first subjected to 100% ethanol, followed by 95%, 70%, and 40% ethanol. Each ethanol wash was done twice for 2 mins before proceeding to the next concentration. Tissue sections were then fully rehydrated using two washes of distilled water (2 mins each). Slides were placed in Hematoxylin 2 stain (Fisher Scientific) for 5 mins. Excess hematoxylin was rinsed from the slide using a gentle stream of running tap water. Slides were then differentiated using 4 quick dips in 0.3% acid alcohol (prepared using 1M HCl and 95% ethanol) for 30 secs. Slides were rinsed well in gently running tap water for 3 mins and then counterstained using an eosin solution for 45 secs. The eosin solution was prepared by dissolving 1% eosin Y (Sigma Aldrich) in distilled water containing 0.5% glacial acetic acid. Slides were then dehydrated by a sequential wash series consisting of 40%, 70%, 95%, and finally 100% ethanol. Ethanol washes were done twice for 2 mins each before proceeding to the next concentration. Slides were then washed twice with xylene (2 mins each) and coverslipped using

Permount mounting medium (Fisher Scientific). Slides were dried for 1 week and observed the next possible lab period.

Lab Period 2

Activity Introduction: In the second lab period, students were introduced to commercially prepared normal bladder tissue slides. Students were asked to study and understand the various tissue layers as a solid understanding is required for accurate disease diagnosis. After students had a strong grasp of healthy tissue, they used their own stained tissues to compare staining proficiency and determine a disease diagnosis/stage.

Evaluation of Normal Bladder Tissue: Students were introduced to bladder tissue layers using H&E stained normal bladder tissue slides (Ward's Scientific). Students were again informed that a solid grasp of the bladder tissue layers is important for disease diagnosis and must be understood before observing their patient samples. To confirm and reinforce their understanding of theses layers, students can list 1-2 defining features for each histological layer and draw a representative image of each layer based on their observations of the normal bladder tissue slide and a provided histology textbook. Once students complete their drawings and describe the features of each layer to the instructor, they can be provided their H&E stained patient samples from the previous week. Students were asked to identify bladder tissue layers within their own samples and identify any abnormalities that might be present by comparing their samples with the normal bladder slide from the commercial vendor.

Diagnosis and Imaging: Students were encouraged to acquire images of tissue regions that appeared pathological. Images can be obtained by placing a cell phone to the oculars of the microscope or by using a dedicated microscopy camera. Representative images of normal and pathological tissues are shown in Figure 1. Students were typically able to diagnose the diseased patient sample as having cancer after observing dense tumors (Figure 1B-C). Normal patient samples show no obvious pathology and should be diagnosed as normal

Disease Staging: Once a cancer diagnosis was obtained, students then determined which stage of bladder cancer was present in their patient sample (Ta, T1, T2b, etc.; See Figure 2 and Table 1). How these stages are determined was not described, which required students to research bladder cancer staging. Students quickly found references to the TNM Staging System (Tumor-Node-Metastasis) developed by the American Joint Committee on Cancer (AJCC) TNM staging is the most commonly used staging system by medical professionals around the world and its guidelines can be easily found on the American Cancer Society website (American Cancer Society, 2016). Although the lymph node

involvement (N) and presence of distant metastases (M) categories cannot be assessed via histological investigation, the invasion of the primary tumor can be determined (T). As illustrated below, the bladder is comprised of various layers (Figure 2A-B) and cancer staging is dependent on which layers the tumor has infiltrated (Figure 2C, Table 1). For example, T2b bladder cancer is diagnosed based on infiltration into the outer muscular layer (Figure 2D), while T3 bladder cancer infiltrates the perivesicual fat layer (Figure 2E).
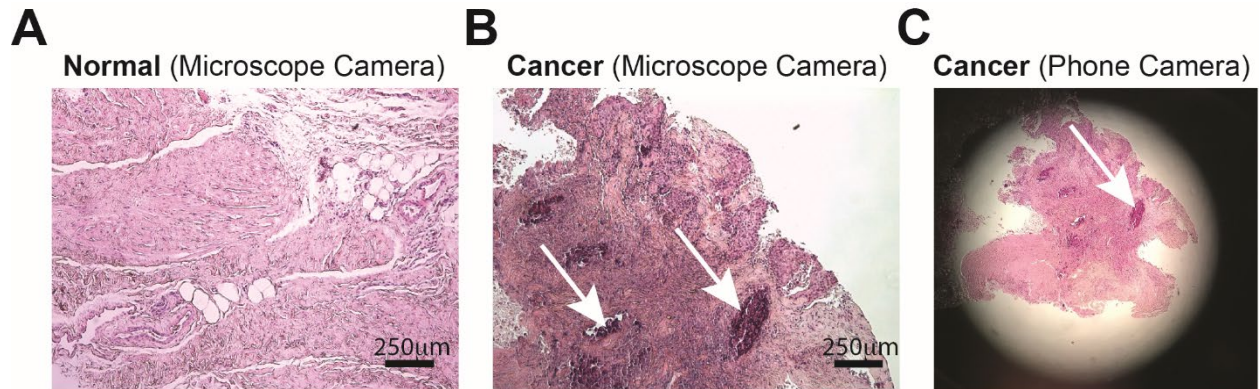
Figure 1. Representative images from normal and cancerous bladder tissue sections. Normal healthy bladder tissue (**A**) has fewer nuclei than cancerous tissue (**B**). The elevated numbers of nuclei in cancerous tissue is indicative of elevated immune cell numbers (i.e., inflammation of the bladder, also known as cystitis). Dense tumors are also apparent in the cancerous tissue, denoted with arrows. Similar images were also obtained using a student's cell phone camera held to the microscope oculars (**C**)
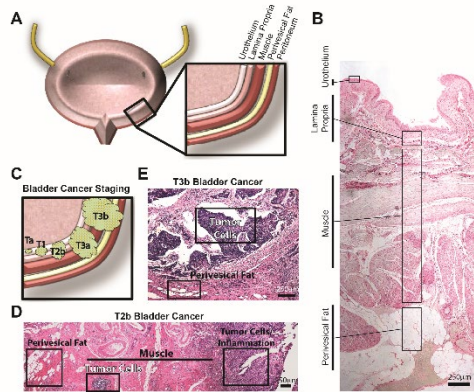
Figure 2. Guidelines for bladder cancer staging. The bladder is comprised of various tissue layers (**A**) that can be identified microscopically following H&E staining (**B**). (**C**) Bladder cancer staging is based on which tissue layers have been infiltrated by the tumor. Shown are examples of H&E stained T2b bladder cancer that is denoted by infiltration into the outer muscular layers (**D**), while T3b bladder cancer is denoted by tumor infiltration into the perivesical fat layer (**E**).

After disease stages were determined, students acquired additional images if needed and emailed their instructor images of the disease features that led to their diagnoses. All student groups can then briefly

present their images to the entire class so that everyone can gain an appreciation for the differences in cancer stages and histological features between patients. Students can also compare their H&E staining technique with professionally stained sections. To do this, one professionally stained adjacent section from the same normal and cancer patien tissues were purchased from the UConn Health Research Biorepository. This activity allowed students to verify that their tissue samples were stained properly.

Stage	Characteristics
Ta	Non-invasive papillary carcinoma
Tis	Non-invasive carcinoma of urothelium
T1	Tumor has invaded connective tissue below urothelium
T2a	Tumor has invaded inner half of muscle
T2b	Tumor has invaded outer half of muscle
T3a	Tumor has spread to outer fat layer; only detected by microscope
T3b	Tumor has spread to outer fat layer; can be seen or felt
T4	Tumor has spread into nearby organs

Table 1. Overview of American Joint Committee on Cancer bladder cancer stages.

Pathology Report: To reinforce the histological findings from this laboratory and have students connect their findings back to their patients, students completed a pathology report. Students were provided guidelines as to what their report should contain, including

Patient ID number.

Symptoms experienced by the diseased patient.

Basic symptoms, which can be found on line, were provided during the first lab period. Symptoms may include severe pelvic pain and/or back pain, problems with frequent urination, painful urination, and bloody urine

Disease diagnosis with corresponding stage.

Histological observations of pathology that support the diagnosis, including how disease staging was determined

Students can discuss observations of inflammation and tumor pathology. Also, students should discuss which layers the tumor infiltrated

A stained and labeled image supporting histological observations and diagnosis.

Students can import their cell phone or microscopy images into PowerPoint so that labels can be added. Visible tissue layers and tissue pathology (e.g., tumor cells) should be labeled. Students should also list which stain they used to visualize the pathology in their tissue samples.

*Depending on the course or content covered, additional pathology report components may include:

Describing nuclear morphology of the tumor cells.

Discussions about possible origins of the tumor (>90% of bladder cancers arise from the urothelium; known as urothelial/transitional cell carcinomas)(Heney, 1992)

After submitting their reports, students compared their conclusions/diagnoses with those prepared by a professional pathologist. Basic pathology reports accompanied all bladder cancer tissues purchased from the UConn Health Research Biorepository.

Safety Considerations: This laboratory involves preserved human tissues. Human tissue should be considered biohazardous and all necessary safety precautions should be used when handling these tissues. All students should wear gloves, lab coats, and protective eyewear. Students should also wash their hands thoroughly upon completion of the lab.

Results

Upon completion of this laboratory, 14 students were polled using anonymous surveys regarding their thoughts about the laboratory. 93% of students considered this activity challenging but appropriate. 86% felt that the activity provided them a greater understanding of pathological tissue. 57% of students reported that they felt they learned tissue layers better with this activity than with traditional textbook and pre-prepared slides used in other laboratories, 35% reported learning the same, while 7% reported that this activity was less effective than traditional approaches. These results suggest that students experienced improved satisfaction and engagement with this activity.

Discussion

Inquiry-based activities are a useful means for stimulating interest, engagement, and problem solving skills (Lord, 2006). The activity described here offers students the opportunity to stain and diagnose diseased human tissue. Students gain real-world experience in common histological techniques (i.e., handling human tissue and H&E staining procedures), a strong knowledge of bladder tissue layers, and insight into cancer pathology and staging. Moreover, pathology labs routinely perform this type of investigation and a complete pathology report is provided with all tissues from the UConn Health Research Biorepository. Thus, students are able to compare their H&E stains and diagnoses with those prepared professionally by a pathologist. By asking students to determine a disease stage, this activity emphasizes the importance of learning different tissue layers for disease diagnosis. In addition to learning tissue layers, students also become aquainted with common pathological hallmarks like inflammation and fibrosis, which are observed while intensely exploring their tissue samples. Students also have the opportunity to observe histological variability between different patients and different cancer stages. Although images may not be necessary for a pathology report, by requiring students to take representative images, students learn about selecting the appropriate parameters to capture features needed for a disease diagnosis (e.g., choosing an appropriate magnification, highlighting diseased tissue, and including tissue boundaries in the image whenever possible). Lastly, while the laboratory described here focuses on a bladder cancer diagnosis, this activity can be applied to a variety of different tissues where cancer staging is dependent on infiltration into defined layers (e.g., colon, esophagus, skin, or stomach cancer).

Acknowledgements

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