VA Puget Sound Surgical Oncology Research Group: Therapy-Induced Senescence and Colorectal Cancer

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Advanced gastrointestinal (GI) cancers are infrequently cured by chemotherapy or radiation, and surgery continues to be the mainstay treatment for most patients resulting in significant impacts on quality of life and survival. Furthermore, the variable responses to cancer treatment and apoptosis markers suggest alternative cell death pathways exist. Our collaborative research group at the VA Puget Sound has been studying and characterizing therapy-induced senescence over the course of several funded projects. Cellular senescence is defined as prolonged cell cycle arrest associated with signs of cell aging. The sentinel work of Hayflick and Campisi revealed that cells will continue to divide until they encounter mortality restrictions at M1/M2 resulting from telomere shortening and activation of p53 and p16/RB pathways. Either or both p53 and p16/RB must be inactivated for immortalization which explains why over 50% of solid tumors harbor mutations at these sites. Malignant cells can be induced into a state of cell cycle arrest following exposure to chemoradiation, termed therapy-induced senescence *(Figure 1)*. Evidence suggests that therapy-induced senescence is a prominent solid tumor response to therapy, and subsets of senescent cells can "escape" and re-enter the cell cycle eventually leading to tumor progression. We observed that massive telomere loss occurs in senescent colorectal cancer cells exposed to chemotherapy. Interestingly, senescent cells able to escape replicative arrest were able to partially recover their telomere loss. We hypothesized that modulation of telomerase activity regulates escape from therapy-induced senescence in colorectal cancer. One of the goals of our lab is to define the role of telomerase in regulating therapy-induced senescence and senescence escape in colorectal cancer. This project is a key component of an ongoing effort to elucidate molecular mechanisms of therapy-induced senescence and identify markers that can reliably predict treatment response and reveal key checkpoints that could be targeted to block senescence escape. A serendipitous finding in our lab of differential adenovirus transfection in senescent cells led to the discovery that coxsackie adenoviral receptor (CAR) expression could be used as a novel marker to quantitate senescence profiles, and a funded pilot study with Nuclear Medicine colleagues to create new radioligand conjugates to detect in vivo senescent tumors with small animal PET imaging. Using CAR as a surrogate biomarker for senescence, we have shown that senescence response can be detected in rectal cancer patients treated with neoadjuvant chemoradiation *(Figure 2)*.

Another project examining CAR expression in archival resected rectal tumor specimens was able to identify nodal micrometastases by routine immunohistochemistry which correlated with patient survival. Recently,

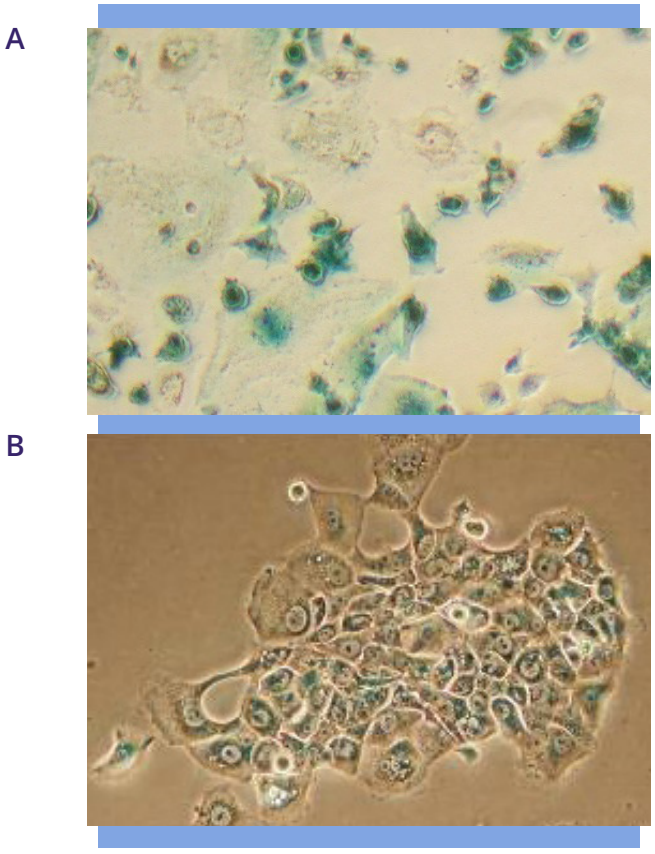

Figure 1. Light microscopy of blue senescent tumor cells in replicative arrest characterized by enlarged and flattened cell shape, increased cytoplasmic granularity, nuclear polyploidy, and expression of pH-restricted SA-beta-galactosidase (A) H1299 lung carcinoma cells. (B) Bx-PC3 pancreatic carcinoma cells.

exosomes have been shown to have potential application as cancer biomarkers with increased sensitivity and specificity compared to traditional protein or nucleic acid markers. Exosomes are 40-50 nm extracellular vesicles released by cells which can embed proteins, lipids and nucleic acids from the parent cell and circulate in human serum. In cancer patients, exosomes are shed from cancer cells and we have been able to measure CAR in tumor-specific exosomes from VA colon cancer patients receiving chemotherapy which allows us to examine cellular events in human patients while undergoing treatment. Our collaborative research group strives towards gaining a better understanding of cancer treatment response, leverage technology to examine cellular events, and develop rational novel targets for clinical testing. An IRB-approved GI Tumor Tissue and Blood Repository is also maintained by the lab group and has accumulated over 250 VA patient samples for research study purposes. Numerous clinical trials are also managed within the group and supported by clinical trial coordinators. Most recently, we received a generous quality improvement grant from the American Cancer Society to improve colorectal cancer screening rates at the VA Puget Sound impacted by the COVID-19 pandemic through the introduction of screening navigators.

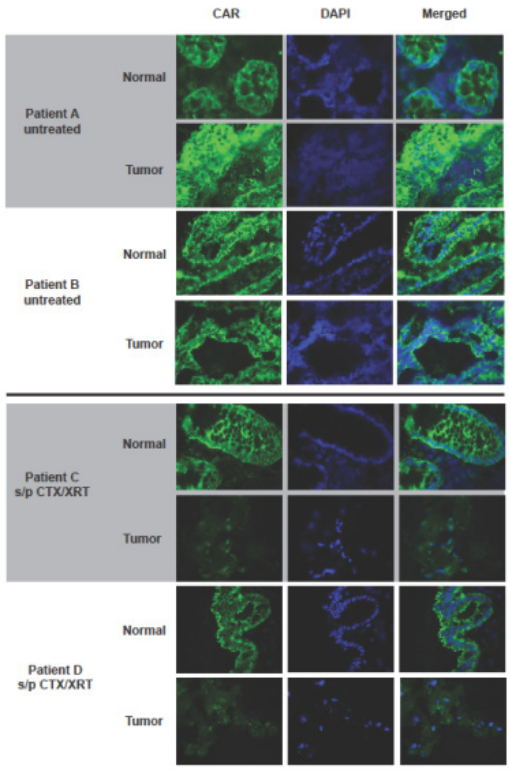

Figure 2. CAR expression and senescence in colorectal cancer. Human tumor and adjacent normal tissue specimens were obtained from colorectal cancer patients undergoing curative resection treated with or without preoperative chemoradiotherapy. Freshly frozen specimens were stained with CAR primary antibody followed with FITC-conjugated secondary antibody or with DAPI. Immunofluorescence micrographs (40X) of CAR, DAPI and merged images are shown.

Research Staff Highlights

Laura Hennessy, RN

Research Nurse Supervisor
Division of Trauma, Burn & Critical Care

How did you get into this line of research?

I was working as a flight nurse with Airlift Northwest and looking for a change. I wanted something that would allow me to learn, teach me a different side of medicine, but allow me to maintain some autonomy. My background has always been trauma and critical care, so it was a pretty easy fit.

What does your typical workday look like/what do you do?

My team and I review all critical care and trauma admissions daily for possible enrollment in our studies. I also am responsible for the regulatory part of research, specifically working with Institutional Review Boards and helping residents and attendings with study designs and submissions. Our studies are either government supported or sponsor supported, so much of my time is working with the budgets and communicating with other sites or sponsors. I also perform research procedures such as blood draws, drug infusions, etc., as well as talk with families and patients about the research.

What has been your most significant accomplishment/finding?

I was once told that as a clinical nurse, I was helping one patient at time, but as a research nurse, I'm contributing to helping the population at large. I hope that the science that has come from our studies will do that for generations to come.

Advice for others looking to get into this field?

I just had my 35th anniversary here at Harborview. I continue to learn from some of the smartest people on the planet. If you want to stay intellectually stimulated and want to feel like you are part of the scientific world, this is the best place to be.