GESINAS - ImmunoTools Award 2023

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Do the effects of oncologic surgery promote a more apoptotic resistant phenotype in tumor cells?

Background and rationale: Previous work has revealed that the effect of oncologic surgery to excise the primary tumor dramatically alters the level of circulating cytokines in the blood of oesophageal adenocarcinoma (OAC) patients as early as 24h post-surgery, returning to baseline within 6 weeks (*Donlon and Davern et al.,).* These effects were characterized by a decrease in the level of circulating anti-tumor cytokines including IFN-y and IL-12 and an increase in the levels of circulating pro-tumor cytokines including IL-6 and IL-8. In this work we also identified that these systemic alterations significantly dampened the cytotoxic ability of lymphocytes to kill OAC tumor cells (*Davern and Gaughan et al.*).

Many OAC patients have a poor response to adjuvant therapies which are therapies given to patients post-surgery. Therefore, we **hypothesize** that the surgery-mediated alteration in systemic circulating cytokine profiles directly decreases the sensitivity of OAC tumor cells to mitochondrial apoptotic cell death, which is an important mechanism of cell death induced by many cancer therapies.

Research Aim: We have previously identified what circulating cytokines are increased immediately post-surgery, therefore, our **specific aim** for this study is to:

Determine if these elevated cytokines directly decrease the sensitivity of OAC tumor cells to mitochondrial apoptosis using a functional precision medicine approach known as dynamic BH3 profiling. If tumor cells are less sensitive to apoptosis then theoretically they would be less sensitive to many drug therapies and immunotherapies. Therefore, the translational findings of this study will provide important clinical insight behind mechanisms of resistance to adjuvant therapies in OAC patients.

Research methodologies: Dynamic BH3 profiling is a fluorescent-based microscopy assay developed by the Letai laboratory that measures the sensitivity of tumor cells to mitochondrial apoptosis following an overnight drug treatment. In this study we will

utilize dynamic BH3 profiling to answer the **research question**: Do the specific cytokines that are increased in systemic circulation post-surgery decrease the sensitivity of tumor cells to apoptosis?

Using previously established protocols in the lab (*Potter et al*.), we will pre-treat tumor cells overnight with 20 different ImmunoTools cytokines which were found to be increased post-surgery in systemic circulation of OAC patients in a previous study (*Donlon and Davern et al.*). The panel of 20 cytokines include: IL-1α, IL-1β, IL-2, IL-4, IL-10, IL-6, IL-8, IL-15, IL-16, IL-22, CRP, CCL22, CTLA-4, MIP-1α, MIP-1β, MIP-3α, PIGF, TNF-α, TSLP and VEGFA.

The next day the cells will be permeabilized and the mitochondria will be exposed to increasing concentrations of the BIM peptide for 1 hour. The percentage of cells that are positive for cytochrome *c* is then measured by fluorescent microscopy. Cells that lose their cytochrome *c* have undergone apoptosis. BIM promiscuously binds to anti-apoptotic proteins, resulting in mitochondrial outer membrane permeabilization (MOMP), cytochrome *c* release and subsequent apoptosis. The more sensitive a cell is to mitochondrial apoptosis the less BIM peptide required to induce cytochrome *c* release and subsequent apoptosis. Conversely, the less sensitive a cell is to apoptosis the more BIM peptide that is required to induce cytochrome *c* release and subsequent apoptosis. This experiment will allow us to determine if the circulating cytokines that are increased in circulation post-surgery also decrease the sensitivity of tumor cells to apoptosis. If this is the case, then tumor cells that remain post-surgery will be less sensitive to apoptosis and theoretically less sensitive to adjuvant therapies such as conventional chemotherapies: 5-FU, oxaliplatin and docetaxel and immunotherapies such as nivolumab, which are administered to OAC patients post-surgery.

Clinical Impact: Drugs known as BH3 mimetics are FDA-approved in haematological malignancies and directly enhance apoptotic sensitivity by inhibiting anti-apoptotic proteins in tumor cells. If we determine that the post-surgical window decreases apoptotic sensitivity in OAC cells via the surgery-mediated alteration in circulating cytokines, this would identify a therapeutic opportunity to administer BH3 mimetics to circumvent these effects. Collectively, this work will provide critical clinical insight behind the lack of therapeutic efficacy in the post-surgical setting and help guide clinical trial design in this therapeutic window to boost the effectiveness of treatments.

Social Commitment: As part of my social commitment, I will partake in “The **Y**oung **E**mpowered **S**cientists for **C**ontin**U**ed **R**esearch **E**ngagement” (YES for CURE) Program hosted by Dana-Farber Cancer Institute and the Harvard Cancer Program. This initiative matches high school and undergraduate students interested in pursuing a career in biomedical research with a mentor that works in the field the student has an interest in. During this program I will mentor a student during a summer research project hosted in our lab. This is a fantastic opportunity for young students to receive one-on-one mentorship from scientists who can provide them with career advice in choosing college courses and to expand their network and open up more career opportunities for them once they leave school. The students also receive training in how to effectively carry out cancer research, gaining experience in research methodologies that would otherwise be unavailable to them and provide them with a unique insight into cancer research.

References

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