Non-small cell lung cancer

Summary

In this thesis, we have investigated the potential of NOTCH signaling as a therapeutic target in non-squamous NSCLC and GBM. We developed novel *in vitro* and *in vivo* models to investigate whether NOTCH inhibition using small molecule inhibitors (GSI) when used in combination with standard of care conventional treatment with chemotherapy and radiotherapy improves treatment response.

Chapter 2 provides a review on the role of NOTCH signaling in the intrinsic and acquired treatment resistance observed in NSCLC. We discuss how the NOTCH signaling pathway is associated with intrinsic mechanism of tumor resistance including its role in: cancer stem cells, ABC drug transporters, epithelial-mesenchymal transition, hypoxia, and crosstalk with other oncogenic pathways such as with TP53, growth factor signaling (e.g. EGFR) tumor angiogenesis (e.g. VEGF) and the DNA damage response (ATM). We conclude that NOTCH-based therapeutic regimens in combination with radiation, and chemotherapy/targeted therapy is a promising strategy to further explore in NSCLC.

In **Chapter 3**, we developed *in vitro* in 2D and 3D semi-high throughput drug testing assays to investigate whether NOTCH inhibition enhanced the anti-tumor effect of chemo- and chemoradiation treatments. We screened 101 FDA approved chemotherapeutics from the NCI library combined with RT and focused on those chemotherapies commonly used as part of first line treatment in NSCLC. Synergistic interactions ($p<0.05$) were observed between the clinical GSI BMS-906024 and chemoradiation (using cisplatin, paclitaxel, docetaxel, and crizotinib). Several combinations treatments were identified were NOTCH blockade enhanced the cytotoxic and RT effect of these treatments.

In **Chapter 4,** we describe the development of an *in vivo* NSCLC tumor xenograft model to test the effect of these single and combined treatments on tumor growth. We identified drug doses of GSI and cisplatin that were effective in tumor growth inhibition but non-toxic. We combined GSI and cisplatin or GSI and RT in tumors with high oncogenic NOTCH activity and those with wildtype NOTCH signaling. Remarkably, we found that H460-NOTCH^{High} tumors are more sensitive than the H460 wildtype model to cisplatin treatment. In tumors with high NOTCH activity we observed increased vessel density, however these vessels appeared non-functional. H460-NOTCH^{High} tumors are more resistant to radiotherapy than H460 wildtype tumors and specific scheduling of GSI with respect to RT resulted in tumor cures for both tumor models.

In **Chapter 5**, the development of an orthotopic NSCLC preclinical model in mice is described. We tested several models and found that H1299 NSCLC most reproducibly produced tumors as a single localized nodule in the lung. We compared two non-invasive imaging modalities; dual wavelength BLI and dual energy CBCT and found that they correlated. Using these models, we illustrate the effect of different tumor volume-based radiation treatment plan set-ups that can be delivered employing an image-guided, high precision small animal irradiator taking into account tumor margins, radiation dose-volume histogram parameters to both the internal and planned target volume and organs at risk (lung, heart, spinal cord).

In **Chapter 6**, we investigated the efficacy of NOTCH inhibitors on glioma tumor growth and tumor resistance *in vitro* and *in vivo*. Using primary and established glioma cell lines and 3D spheroid models we demonstrate that NOTCH signaling was active but that NOTCH inhibition alone was insufficient to block tumor cell survival. Next, we combined clinically approved NOTCH/γ-secretase inhibitor with the standard of care treatment for glioblastoma: concurrent alkylating chemotherapy with temozolomide and radiotherapy. We also tested these combination treatments in an intracranial model from GBM using U87 cells and tested NOTCH inhibitors alone or in combination with standard of care treatment and monitored tumor growth response using non-invasive imaging. We found that similar to our results *in vitro*, NOTCH inhibition alone did not significantly block tumor growth but that when combined with temozolomide, radiotherapy or both, it significantly prolonged survival. *In vitro* and *in vivo* NOTCH inhibition blocked the survival of treatment resistant CD133 cancer stem cells.

Finally, **Chapter 7** summarizes the findings of this thesis and comments on the future challenges and opportunities for NOTCH-based interventions in cancer.