Preclinical Modeling of Novel Therapeutics in Patient Derived Xenografts of Solid Tumors

by

Amelia S. Zessin

Department of Molecular Cancer Biology
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Date: ________________________

Approved:

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David Hsu, Supervisor

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David Kirsch

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Kris Wood, Chair

Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the Department of Molecular Cancer Biology in the Graduate School of Duke University

2015
ABSTRACT

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Abstract

Due to the failure of many drugs in the transition from the preclinical setting into clinical trials, better mouse models of cancer are needed. To this end, our lab has created a patient derived xenograft (PDX) system by processing tumors as they are resected from the patient and directly injected them into immunodeficient mice without the intermediate step of cell culture. This allows us to work with tumors that are very similar to the original patient tumors, unlike cell lines, which have many alterations before they are used for xenograft work. Our PDX model therefore is ideal for preclinical modeling to study novel therapeutics in vivo and we have applied our model to answer two clinically important questions; 1) How can we identify and characterize actionable mutations in cancer and 2) What is the effect of exercise on tumorigenesis?

One current difficulty clinicians in oncology have is directing patients to the therapy that will be most effective with minimal toxicity. Next-generation sequencing platforms address this problem by providing clinicians an overview of their patient’s mutation status. However, it is not known, the most cost effective sequencing platform that gives sufficient data to properly direct a patient’s therapy and which mutations are actionable. Therefore, I tested two different sequencing panels comprising of 50 genes (Ion AmpliSeq Cancer Hot Spot Panel) and more than 400 genes (Ion AmpliSeq Cancer Comprehensive Cancer Panel) to determine how many genes need to be sequenced to
sufficiently identify actionable mutations. Next, to determine if mutations are targetable, I directed PDXs into therapy based upon the results from each of the panels. I determined that the 50 gene panel was not sufficient to identify actionable mutations, while although the 400 gene panel provided mutation data to guide therapy, many of the mutations are not targetable and thus require further testing.

Exercise is a potential therapy that has recently been shown to be potentially effective in mouse models of cancer, as well as decreasing time to progression for cancer patients. However, it is unclear how exercise therapy decreases the growth of tumors. To explore this in detail, I used our PDX model and investigated whether three different colon cancer PDXs (CRC240, CRC282, CRC370) would respond to exercise. I found that CRC282 and CRC370 responded to exercise, as evidenced by decreased tumor growth. To investigate the mechanism of the effects of exercise therapy, I used gene set enrichment analysis and found that markers of hypoxia are decreased in tumors of mice that have exercised compared to those that were sedentary during the experiment. Using immunofluorescence, we confirmed that there is a decrease in hypoxia in tumors of mice that have exercised, providing a possible explanation for the decrease in tumor growth in mice that exercised compared to those that are sedentary.
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1. Next-Generation Sequencing as a Tool for Screening Potential Novel Therapeutics

1.1 Patient Derived Xenografts

Prior to the incorporation of new therapeutic agents and to study tumor biology in the clinical setting, drugs must be assessed for their therapeutic potential in predictive preclinical models. To this end, mouse xenografts have been developed to screen new cancer drugs (Suggitt & Bibby, 2005). Initially, athymic mice (nu/nu) and severe compromised immunodeficient (SCID) mice were used to establish xenografts from human tumor cell lines to test their response to cancer drugs (Alley, Hollingshead, Dykes, & Waud, 2004). More recently, the direct transplantation of resected human tumors into mice to study sensitivities to therapeutic agents in gastrointestinal cancers has been performed (Pitts et al., 2010; Rubio-Viqueira et al., 2006).

In order to develop a preclinical model to study novel drug combinations, we are currently collecting resected tumor samples at the time of surgery under a Duke approved IRB protocol (Pro00002435). These samples are then injected into the flanks of non-obese diabetic–severe combined immunodeficient (NOD-SCID) mice to generate patient derived xenografts (PDXs) (Kim et al., 2012; Uronis et al., 2012). Unlike cell line xenografts, tumors grown using this method retain the architecture of the patient tumors, including supporting stromal components (Figure 1). Additionally, when gene expression is analyzed using unsupervised clustering, the original patient tumor and the explant most often cluster together, indicating that there are little changes in gene
expression that occur when growing patient tumor chunks in mice (Uronis et al., 2012). As a result, the availability of these PDXs provides a powerful tool to study tumor biology to discover and characterize new and novel therapeutic targets.

![Image](image.png)

**Figure 1: Generation of Patient Derived Xenografts.** Patient derived xenografts of solid tumors (bottom left) maintain stroma and morphology of original patient tumor (top left). Xenografts derived from cell lines (right) do not maintain original tumor structure. Adapted from Uronis et al 2012.

Next-generation sequencing is a powerful tool for finding large numbers of mutations in tissue samples that may potentially direct patients into treatment groups. However, sequencing a patient’s entire genome is costly and also provides a large dataset that can be difficult to interpret. In order to keep costs low and provide physicians with meaningful data, companies such as Ion Torrent have developed sequencing panels specific to cancer related genes and mutations. These panels are the
Ion AmpliSeq Cancer Hotspot Panel and Comprehensive Cancer Panel. The Hotspot Panel uses high throughput sequencing to determine the mutation status of over 200 mutations in 50 genes that are commonly associated with cancer. The Comprehensive Cancer Panel uses the same methods as the Hotspot Panel but gives whole exome coverage of over 400 cancer related genes.

1.2 Sequencing a panel of patient tumors and patient derived xenografts using a Hotspot Panel

To determine the minimum amount of sequencing required to direct patients towards potentially effective therapeutics, we first sequenced twelve patient tumors with matched patient derived xenografts (Table 1). Samples submitted for DNA isolation and sequencing had greater than 50% tumor, as analyzed by a pathologist. Mutations were called based on 100X coverage and 25% frequency, allowing for the calling of heterozygous mutations within the tumor. Results of sequencing are shown in Figure 2.

Of the mutations found, two were potential novel therapeutic targets within their respective tumor type. The first was an ERBB2 mutation within CRC240, a PDX of colon cancer. The second was an IDH1 mutation within CRC292, a PDX of cholangiocarcinoma. Other targetable mutations include BRAF V600K in a sample of melanoma, which has been previously described to be sensitive to Vemurafenib, a small molecule inhibitor used to treat BRAF V600E mutant melanoma (Lee et al., 2010; Rubinstein et al., 2010).
Table 1: Samples Sequenced in Hotspot Panel. Identification, site of primary tumor, and metastatic site (if applicable) of tumors sequenced using the Hotspot panel.

<table>
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<tr>
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<th>Metastatic Site</th>
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<td>MLA005</td>
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Figure 2: Mutations Identified by Hotspot Cancer Panel. Fifty common tumor suppressor genes and oncogenes were sequenced in ten patient tumor samples and corresponding patient derived explant samples utilizing the Ion AmpliSeq Cancer Hotspot Panel v2. Called mutations (>25% call rate in both patient and PDE) are highlighted in red (nonsense mutation), yellow (missense mutation), or blue (frameshift mutation). CRC = colorectal cancer; CHOL = cholangiocarcinoma; LUNG = lung cancer, MLA = melanoma.
1.2.1 Isocitrate dehydrogenase inhibition

Isocitrate dehydrogenase 1 (IDH1) is a metabolic protein commonly mutated in glioma and acute myeloid leukemia (Yan et al 2009, Abass et al 2010). Recently, studies have shown IDH1 to be mutated in p.R132 in about 25% of intrahepatic cholangiocarcinoma samples (Kipp et al 2012, Borger et al 2012). The mutation of the arginine at residue 132 alters the binding of IDH1 to its substrate, isocitrate, and weakens the conversion to α-ketoglutarate (Guerra et al 2009). Additionally, the mutation causes a gain-of-function in the IDH1 protein and allows for the conversion of α-ketoglutarate to 2-hydroxyglutarate (Dang et al 2009), which has been shown to be an oncometabolite with the ability to transform human epithelial and blood cells into cancerous cells (Koivunen et al., 2012; Losman et al., 2013).

AGI-5198 is an inhibitor recently developed by Agios Pharmaceuticals and has been shown to specifically inhibit IDH1 mutated at p.R132 (Popovici-Muller et al 2012, Rohle et al 2012). By inhibiting only the mutant form of the protein and not the wild type form, the production of α-ketoglutarate is maintained while the conversion to 2-hydroxyglutarate is ceased. Because 2-hydroxyglutarate acts as a competitive inhibitor of α-ketoglutarate, ceasing the production of 2-hydroxyglutarate allows for the activation of α-ketoglutarate dependent proteins, such as TET2 DNA demethylase, Jumanji histone demethylases, and prolyl hydroxylases.
Glioma cells that are IDH1 R132H mutant are sensitive to treatment in vitro using colony formation assays, and in vivo using tumor growth inhibition (Rohle et al 2012). To determine if CRC292, the cholangiocarcinoma sample found to be IDH1 R132C mutant in the Hotspot panel, is sensitive to AGI-5198 treatment, we treated CRC292 with AGI-5198 in vitro and in vivo (Figure 3). There was no effect on the viability of CRC292 cells when treated with AGI-5198 for 48 hours in vitro (Figure 3a). This corresponded with no change in the levels of 2-hydroxyglutarate within the cells, as assessed by LC/MS/MS (Figure 3b). To determine if AGI-5198 is effective at inhibiting tumor growth in vivo, CRC292 PDX was homogenized and injected subcutaneously into the flank of NOD-SCID mice. Once tumors formed, mice were treated daily for three weeks with 150 mg/kg AGI-5198 or vehicle through oral gavage. Contrary to the effects of treatment in vitro, AGI-5198 treatment in vivo caused a significant decrease in tumor growth over three weeks (Figure 3c). When lysates from treated and untreated PDXs were analyzed for 2-hydroxyglutarate levels, AGI-5198 treatment decreased the amount of 2-hydroxyglutarate within the tumor (Figure 3d). Further studies are ongoing to determine why AGI-5198 is ineffective at inhibiting the growth of CRC292 cells in vitro but is effective at inhibiting tumor growth and the production of 2-hydroxyglutarate in vivo.
Figure 3: *In vitro and in vivo* Treatment of CRC292 with AGI-5198. CRC292 was treated *in vitro* and *in vivo* with AGI-5198, an inhibitor of mutant IDH1, predicted to be effective due to mutations identified in the Hotspot Cancer Panel (Figure 1). a. CRC292 was treated *in vitro* with escalating doses of AGI-5198, an inhibitor of mutant IDH1, for 48 hours. Data is represented as the the fold change in RLU using Cell Titer Glo compared to vehicle (n=4 per dose). b. CRC292 was treated with escalating doses of AGI-5198 for 48 hours. After treatment, cells were spun down and submitted for 2-HG measurement by LC/MS/MS (n=3 per dose). c. CRC292 was treated daily with vehicle (n=6) or 150 mg/kg AGI-5198 (n=8), for 21 days (**p<0.005). Tumors were measure two times per week. d. Tumors were harvested from mice 6 hours after the final treatment and submitted for 2-hydroxyglutarate analysis by LC/MS/MS (*p<0.05).

1.2.2 Her2 inhibition

Her2, encoded by the ERRB2 gene, is commonly upregulated in breast cancer and is targeted by Herceptin, an antibody treatment commonly used in breast cancers with amplification of ERBB2 (Ross & Fletcher, 1998). ERBB2 mutations have not been previously described in colon cancer as a marker for sensitivity to Her2 inhibition.
However, insertions within the gene at the 775\textsuperscript{th} residue have been shown in NSCLC to activate the Her2 protein and increase kinase activity (Wang et al., 2006). Since our mutation in ERBB2 corresponded to the 776\textsuperscript{th} residue of the Her2 protein, we tested CRC240 both \textit{in vitro} and \textit{in vivo} to determine if Her2 inhibition is a potential treatment for patients with Her2 G776V mutant colon cancer.

\textit{In vitro}, we exposed CRC240 and BT474 cells to Herceptin. BT474 is a Her2-positive breast cancer cell line that is sensitive to Her2 inhibition. Cells were treated with 20 µg/µl of Herceptin for 48 hours and viability was determined by Cell Titer Glo analysis. Compared to BT474, CRC240 was resistant to Herceptin treatment and did not show any growth inhibition compared to cells treated with vehicle (Figure 4a). Western blot analysis indicated that this is due to a lack in the expression of the Her2 protein within CRC240 (Figure 4b).

Because we have seen differences in the response of cell lines and PDXs to drug treatments, we also tested CRC240 with Herceptin \textit{in vivo}. CRC240 PDXs were injected subcutaneously as a homogeneous suspension into NOD-SCID mice and monitored for growth three times per week. Once tumors reached a volume of 200 mm\textsuperscript{3}, mice were randomized into vehicle or Herceptin treatment groups. Herceptin was administered twice per week IP at 0.3 mg/kg. Results from Herceptin treatment of CRC240 \textit{in vivo} are shown in Figure 4c. Similar to \textit{in vitro} results, CRC240 explants were not sensitive to Herceptin treatment.
Figure 4: *In vitro and in vivo* Treatment of CRC240 with Herceptin. CRC240 was predicted to respond to treatment with Herceptin due to a mutation found in Her2 using the Hotspot Panel (Figure 1). 

a. CRC240 and BT474 (Her2 amplification positive) cell lines were treated with 20 μg/μl of Herceptin for 0 or 48 hours and analyzed using Cell Titer Glo (n=4 per group). 

b. Unlike BT474, CRC240 does not have detectable levels of total or phosphorylated Her2. 

c. CRC240 was treated twice per week with vehicle (n=5) or 3 mg/kg Herceptin (n=5), an inhibitor of Her2, for 21 days.

1.3 Sequencing patient derived tumor xenografts using the *Comprehensive Cancer Panel*

Because most of the findings when using the Hotspot Panel would not be sufficient to direct patients in to therapy, we resequenced two patient tumors and their corresponding PDXs using the Comprehensive Cancer Panel from Ion Torrent. The Comprehensive Cancer Panel provides whole exome sequencing of over 400 cancer related genes, allowing for the identification of more potential markers of drug
sensitivity. The two tumors sequenced were CRC292, a cholangiocarcinoma, and BRPC 12-146, a colorectal carcinoma.

In order to prioritize findings from the Comprehensive Cancer Panel, we used transFIC software, which provides transformed functional impact scores for mutations using pathway analysis and germline tolerance of the mutation. Pathway analysis is used to rank those mutations in proteins in pathways known to drive cancer higher than those in proteins in pathways that have little to no evidence of involvement within cancer. Germline tolerance is determined by searching 1000 Genomes for the prevalence of that particular mutation in normal tissue. Those SNPs commonly found in 1000 Genomes are given lower impact scores due to the probability that they are not cancer drivers.

After running the sequencing data through transFIC, we sifted through potential driver candidates for those proteins with a small molecule inhibitor available. After filtering through all the mutations, CRC292 had 10 mutations that were potentially targetable and BRPC 12-146 had 18 mutations (Table 2). We decided to treat both CRC292 and BRPC 12-146 with the same drug panel that targeted mutations found in both samples. The drug panel consisted of the following four drugs: Sunitinib, Trastuzumab, GNF-5, and Ruxolitinib.

Table 2: Potential actionable mutations found through the Comprehensive Cancer Panel and transFIC. Whole exons of 409 cancer related genes were sequenced in CRC292 (a) and BRPC 12-146 (b) using the Ion AmpliSeq Comprehensive Cancer Panel. Called mutations (>25% frequency and >100x coverage) were analyzed using
transFIC (bg.upf.edu/transfic) to assess their potential impact in cancer. Mutations with high average transFIC scores (>1.83, red) and medium average transFIC scores (between -1 and 1.83, yellow) were filtered for oncogenes with targeted inhibitors.

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### 1.3.1 Treatment of two patient derived xenografts with Sunitinib

Both CRC292 and BRPC 12-146 had mutations in CSF1R and Flt4 that are each targeted by Sunitinib. Additionally, CRC292 had mutations in VEGFR2 and Flt3 that are targeted by Sunitinib. To determine the sensitivity of each xenograft to Sunitinib treatment, the tumors were homogenized and injected subcutaneously in to NOD-SCID mice. Tumors were allowed to grow to 200 mm³ and mice were randomized into control or treatment groups. Sunitinib treatment mice received 40 mg/kg of Sunitinib through oral gavage daily whereas control mice received the same relative volume of vehicle. The growth of the xenografts during treatment is shown in Figure 5. CRC292 was highly sensitive to Sunitinib treatment, as the tumors within the treatment group stopped growing after treatment began and remained around 200 mm³ (Figure 5a). Additionally,
the weights of the Sunitinib treated tumors were significantly decreased compared to the weights of tumors in the vehicle treated group (Figure 5b). BRPC 12-146 was slightly sensitive to Sunitinib treatment, as shown by a delay in growth and by a significant decrease in the final weights of the tumors (Figure 5c, d). However, the growth curves of the treated and untreated tumors were not significantly different, most likely due to a small sample size and the variability of growth within the vehicle treated group (Figure 5c).

Figure 5: Sunitinib treatment of CRC292 and BRPC 12-146. a. Treatment of CRC292 for 21 days with vehicle (n=6) or Sutent (40 mg/kg daily, n=6) (**p<0.005, paired t-test). b. Tumor weights after 21 days of treatment with Sutent (**p<0.0001, t-test). c. Treatment of BRPC 12-146 for 21 days with vehicle (n=4) or Sutent (40 mg/kg daily, n=4) (n/s, paired t-test). d. BRPC 12-146 tumor weights after 2.5 weeks of treatment with Sutent(**p<0.005, t-test).
Sunitinib is a multi-target tyrosine kinase inhibitor so it is unclear which mutation, if any, is causing sensitivity to Sunitinib. To investigate further, I performed Western blot analysis or immunohistochemistry on targets of Sunitinib that were mutated in either sample. The first target I tested was CSF1R, which was mutated in both CRC292 and BRPC 12-146. To determine the effect of Sunitinib treatment on the total amount of CSF1R, I stained six vehicle and six Sunitinib treated CRC292 tumor samples (Figure 6). When quantifying the percent staining of total CSF1R in each sample, the amount of protein is slightly decreased in the Sunitinib treated samples compared to the vehicle treated samples, however this effect is not significant (Figure 6). It is not yet known whether or not the amount of active CSF1R is affected upon treatment with Sunitinib.

![Immunohistochemical staining for CSF1R in vehicle and Sunitinib treated CRC292 tumors. Quantification on the right is from a total of six samples per group with one field per sample.](image)

Figure 6: Immunohistochemical staining for CSF1R in vehicle and Sunitinib treated CRC292 tumors. Quantification on the right is from a total of six samples per group with one field per sample.
Another target of Sunitinib mutated in CRC292 is KDR, or VEGFR2. To determine the effect of Sunitinib on the activity of VEGFR2, I stained six vehicle treated and six Sunitinib treated tumor samples and submitted them to a pathologist for analysis. When comparing vehicle and Sunitinib treatment, there was no difference in the levels of total or phosphorylated VEGFR2, though this may be because of a low concentration of antibody (data not shown).

A third possible mutation that correlates with Sunitinib sensitivity in CRC292 is Flt3. To determine the effect of Sunitinib treatment on the levels of total and phosphorylated Flt3 in CRC292, I used Western blot analysis, as shown in Figure 7. The levels of both total and phosphorylated Flt3 were inconsistent within groups, however, the levels of each are so low that is unlikely that mutant Flt3 is the cause of the Sunitinib sensitivity of CRC292.

![Western blot analysis of Flt3 (phospho-Y591 and total) in vehicle and Sunitinib treated CRC292. Mice were treated as described above and tumors were harvested at the end of three weeks of treatment.](image)

Figure 7: Western blot analysis of Flt3 (phospho-Y591 and total) in vehicle and Sunitinib treated CRC292. Mice were treated as described above and tumors were harvested at the end of three weeks of treatment.
1.3.2 Treatment of two ABL2 mutant patient derived xenografts with GNF-5

Both BRPC 12-146 and CRC292 had I256F mutations in ABL2. GNF-5 is a small molecule inhibitor that targets Abl family members Abl and Arg (Abl2). To determine if ABL2 I256F sensitizes tumors to GNF-5 treatment, I injected BPRC 12-146 or CRC292 subcutaneously into mice and allowed tumors to grow. Once tumors reached a volume of ~200 mm³, mice were divided into vehicle or GNF-5 treatment groups. GNF-5 treatment was administered IP daily at a dose of 50 mg/kg. After three weeks of treatment, neither BRPC 12-146 nor CRC292 showed a response to GNF-5 treatment, as shown in Figure 8. Therefore, ABL2 I256F alone does not correlate with sensitivity to GNF-5 treatment.

Figure 8: Treatment of CRC292 (a, b) and BRPC 12-146 (c, d) with GNF-5. PDXs were homogenized and injected subcutaneously. Once tumors reached 200 mm³, mice
were treated with IP GNF-5 at 50 mg/kg daily for 3 weeks. Tumors were measured three times per week and weighed at the completion of the experiment.

1.3.3 Treatment of two ERBB2 mutant patient derived xenografts with Herceptin

ERBB2 P1170A mutations were discovered in both CRC292 and BRPC 12-146, indicating that the tumors may be sensitive to Herceptin, an antibody that targets the Her2 protein (encoded by the ERBB2 gene). The mutation has not been previously described so it is unknown if it causes activation of the protein. To determine if the mutation causes sensitivity to Herceptin, we injected either CRC292 or BRPC 12-146 in mice and randomized them into vehicle or Herceptin treatment groups once tumors grew. After three weeks of treatment, Herceptin had no effect on the growth of BRPC 12-146 or CRC292, indicating that a P1170A mutation in ERBB2 does not correlate with sensitivity to Herceptin (Figure 9).
2.6 Figure 9: Treatment of CRC292 and BRPC 12-146 with Herceptin. Mice were injected with homogenized CRC292 (a, b) or BRPC 12-146 (c, d) and treated with twice per week with 0.3 mg/kg IP Herceptin. Tumors were measure three times per week and tumors were weighed upon completion of the experiment.

1.3.4 Treatment of two JAK mutant patient derived xenografts with Ruxolitinib

Ruxolitinib is a small molecule inhibitor that targets the JAK/STAT pathway. Because both BRPC 12-146 and CRC292 have JAK2 mutations, we hypothesized that they may be sensitive to treatment with Ruxolitinib. The mutation has not been previously described so it is unknown if it causes and increase in activity of the JAK/STAT pathway. To determine the efficacy of Ruxolitinib treatment, we injected BRPC 12-146 or CRC292 tumors into mice and randomized mice into control or Ruxolitinib treatment groups. Ruxolitinib was given in a chow, which mice were able to
access *ad libitum*, and control mice were given a control chow that did not contain the drug. After three weeks of treatment, CRC292 tumor growth was significantly decreased, whereas BRPC 12-146 tumor growth was unchanged (Figure 10).

![Graphs of CRC292 and BRPC 12-146 tumor volumes and weights under treatment with Ruxolitinib or Vehicle.](image)

Figure 10: Ruxolitinib treatment of CRC292 (a, b) and BRPC 12-146 (c, d) *p<0.05.

**1.4 Conclusions**

Because of the cost and effort associated with sequencing, it is important to determine the minimum amount of sequencing required to direct patients into the appropriate treatment groups. We determined that for most patients, the Hotspot panel would not be sufficient as most patients did not have targetable mutations. The Comprehensive Cancer Panel may be sufficient to direct patients into treatment but mutations must be validated for sensitivity to drugs before determining if the treatment
will be right for the patient. Further work is needed to determine predictive biomarkers and the most efficient way of screening patients and directing therapy.
2. Exercise as a Cancer Therapeutic

Exercise has been shown to decrease the growth of mouse tumor xenografts and be effective in preventing recurrence of tumors after chemotherapy in breast and colon cancer survivors (Galanti, Stefani, & Gensini, 2013; Higgins, Park, Lee, Curran, & Deng, 2014). Previous work done by the lab of Mark Dewhirst has indicated that this may be because of the formation of more mature blood vessels and decreases in hypoxia leading to a less aggressive tumor phenotype (Betof et al., 2015). However, it is not known how exercise affects gene expression of tumors.

2.1 Panel of patient derived colon cancer explants on exercise therapy

To determine if exercise therapy is useful in the growth inhibition of colorectal cancer patient derived xenografts (PDXs), we used a panel of three colon cancer PDXs. Mice were injected subcutaneously with homogeneous suspensions of the PDX and were monitored until tumor formation began. Once mice had visible tumors, they were randomized into sedentary or exercise treatment groups. Mice in the sedentary group were singly housed with an enrichment hut. Mice in the exercise group were singly housed with a wheel. During the initial pilot experiment, wheels were not able to be monitored for distance and speed. However, in follow up experiments, each mouse in the exercise group was housed with a wheel with a magnetic sensor to determine the distance each mouse travelled daily during the course of the experiment. Tumors were
measured three times per week. Survival curves were determined using time to 1000 mm$^3$ as an endpoint.

Of the three explants tested, one was resistant (CRC240) and two were sensitive (CRC370, CRC282) to exercise therapy, as seen in Figure 11. The resistant PDX, CRC240 had a faster growth rate (>2000 mm$^3$ in 50d) than CRC370 or CRC282 PDX (Growth rate 1000 mm$^3$ in 50d and 80d respectively). This would suggest that patients with aggressive and fast growing tumors may not benefit from exercise therapy as much as those with slower growing tumors.
2.2 Gene expression analysis of exercise treated explants

To identify potential mechanisms of growth inhibition when mice are allowed to exercise, we obtained gene expression data on three sedentary and three exercise samples from tumors of each of the three explants using the Affymetrix U133 array. Using gene set expression analysis (GSEA) to identify biological processes and pathways that were differently regulated between exercise and sedentary mice (Subramanian et al., 2005). Of note, genes downregulated in response to hypoxia tended to be upregulated in
tumors of mice that had access to a wheel, indicating that hypoxia may be decreased in response to exercise treatment. We confirmed this by analyzing the amount of hypoxia within the tumors of exercise mice compared to those of sedentary mice using EF5 staining. EF5 marks areas of tissue that do not have oxygen present allowing for quantification of hypoxia within tumors. Mice were injected with EF5 3 hours prior to sacrifice and tumors were imaged using an antibody for EF5. The amount of EF5 staining per section was quantified as shown in Figure 12. Hypoxia was decreased in tumors of mice that had been given a wheel compared to those that were sedentary. The effect is not significant, though this is most likely a consequence of the sample size (n=3 per group).

Figure 12: Quantification of hypoxia in CRC370 tumors. Representative staining of EF5 (purple) and Hoechst (blue) in sedentary and wheel exercised mice (a) and quantification of the percent hypoxia in the tissue from 3 sedentary and 3 exercised mice (b).

2.3 Conclusions

Exercise therapy is effective in some PDX models, particularly those that are slow growing. After exercise therapy, hypoxia is decreased in comparison to tumors
from mice that are sedentary. This is also demonstrated by the decrease in gene expression of genes associated with hypoxia. More work is needed to determine if there are biomarkers of resistance or sensitivity to exercise therapy. Additionally, the decrease in hypoxia may increase sensitivity to radiation and chemotherapy and experiments are ongoing to determine the effect exercise has on these therapies.
References


