Cancer Biology Graduate Program

Part I Qualifying Exam

June 1, 2018

Please use the paper provided to write out your answers. You must answer 8 of the 10 questions.

To pass you must score 70% overall and 7 or higher on 6 questions.

Write the question number at the top left, and your student number on the top right of the first page of each answer.

Please be concise and limit your answers to what has been asked. Do not include extraneous information in your answers.

Good Luck!

Question 1: (Sumin Kang)

Tumor metastasis, a major contributor to deaths from nearly all types of cancers, is a multi-step cascading process that is influenced by a number of cell signaling proteins. Initiation of metastasis requires invasion. In addition, metastatic tumor cells must acquire migratory potential and resistance to anoikis (extracellular matrix detachment-induced apoptosis) to survive during circulation before forming metastatic foci in distant organs. Through a genome-wide expression profiling comparing circulating tumor cells to primary tumor cells, your lab recently identified that expression of the gene for an enzyme called N-acetyltransferase 2 (NAT2) is increased in disseminated breast cancer cells.

a. (3 points) Design two cell line-based *in vitro* experimental approaches and one *in vivo* xenograft model study to test whether NAT2 plays an important role in maintenance of invasive, anoikis resistant, and metastatic properties of breast cancer cells. Interpret the anticipated result.

- *In vitro* experimental approaches: cell invasion assay and anoikis assay using breast cancer cells with NAT2 inhibition by NAT2 shRNA/sgRNA or NAT inhibitors.
- *In vivo* xenograft experiment using breast cancer cells with NAT knockdown cells or inhibitor treatment either experimental metastasis model (tail vein injection) or spontaneous metastasis model (subcutaneous injection). Metastasis will be decreased in mice xenografted cells with NAT2 inhibition, if NAT2 is critical for metastasis.

b. (2 points) You performed a proteome-wide acetylation profiling in breast cancer cells with NAT2 knockout to identify any potential downstream effectors to confer anoikis resistance or invasive potential and found that the acetylation level of matrix metallopeptidase 1 (MMP-1) at lysine 72 in cells are dramatically decreased when you eliminate NAT2 in breast cancer cells. Design one set of *in vitro* and *in vivo* experiment to examine whether NAT2 promotes invasion and/or anoikis resistance *in vitro* and tumor metastasis *in vivo* by signaling through MMP-1 at K72 in breast cancer cells.

Rescue express MMP-1 variants including acetylation-deficient mutant (K72R) or acetylation-mimetic mutant (K72Q) in NAT2 target downregulated cells and see whether K72Q but not K72R mutant form of MMP-1 can rescue the decreased invasion/anoikis resistance and metastasis caused by NAT2 knockout *in vitro* and *in vivo*, respectively.

c. (2 points) You wish to explore how NAT2-mediated acetylation of MMP-1 regulates MMP-1 to promote invasion and/or anoikis resistance in breast cancer cells. Considering that MMP-1 is involved in proteolytic cleavage in cancer cells, design an *in vitro* study to explore the molecular mechasnim how acetylation of MMP-1 at K72 by NAT2 contributes to proteolytic activity of MMP-1. Interpret the anticipated result.

Induce acetylation by active recombinant NAT2 using purify recombinant MMP-1 WT and K72R, as substrates. Use these acetylated MMP-1 variants and check MMP-1 activity. If acetylated MMP-1 WT but not K72R has higher MMP-1 proteolytic activity, this suggests that MMP-1 acetylation at K72 promotes its activity leading to invasion/anoikis resistance.

d. (3 points) You identified that NAT2 signals through MMP-1, which provides pro-invasive and anti-anoikis property to breast cancer cells to metastasize. You have a panel of primary and lung metastasized paired breast cancer patient tumor tissues. Design three correlation studies you would perform to clinically validate your functional finding.

- Do immunohistochemistry staining of NAT2 and MMP-1 K72 acetylation. Check whether there is any positive correlation between:
    - NAT2 vs metastasis free survival/overall survival (OS),
    - MMP-1 K72 acetylation vs metastasis free survival/OS, and
    - Whether there is any positive correlation between expression or activation of NAT2 vs acetylation of MMP-1.

Question 2: (Bernard Mainou)

a. (6 points) You've discovered a new virus that you have named hawkavir. You are intrigued about the ability of hawkavir to act as an oncolytic virus. When you add hawkavir to normal epithelial cells, the virus establishes infection poorly and the cells remain viable. In contrast, when you add hawkavir to transformed epithelial cells the virus infects the cells efficiently and rapidly induces programmed cell death.

Name two features that might help hawkavir more efficiently infect and kill cancer cells? (Note that there may be more than two possible features of interest but you should pick two and only two to discuss in your answer). Describe an experiment test if each of these two features is involved in hawkavir enhanced cytopathicity in cancer cells.

- Differential receptor expression (test differential cell surface expression of receptor)
- Enhanced cathepsin/proteolytic expression (cathepsin levels being higher on transformed cells)
- Decreased antiviral state (impaired IFN production)
- Altered metabolic state (viral replication under specific metabolic environments like hypoxia)
- Cycling cells (lack of replication in cell cycle arrested cells)

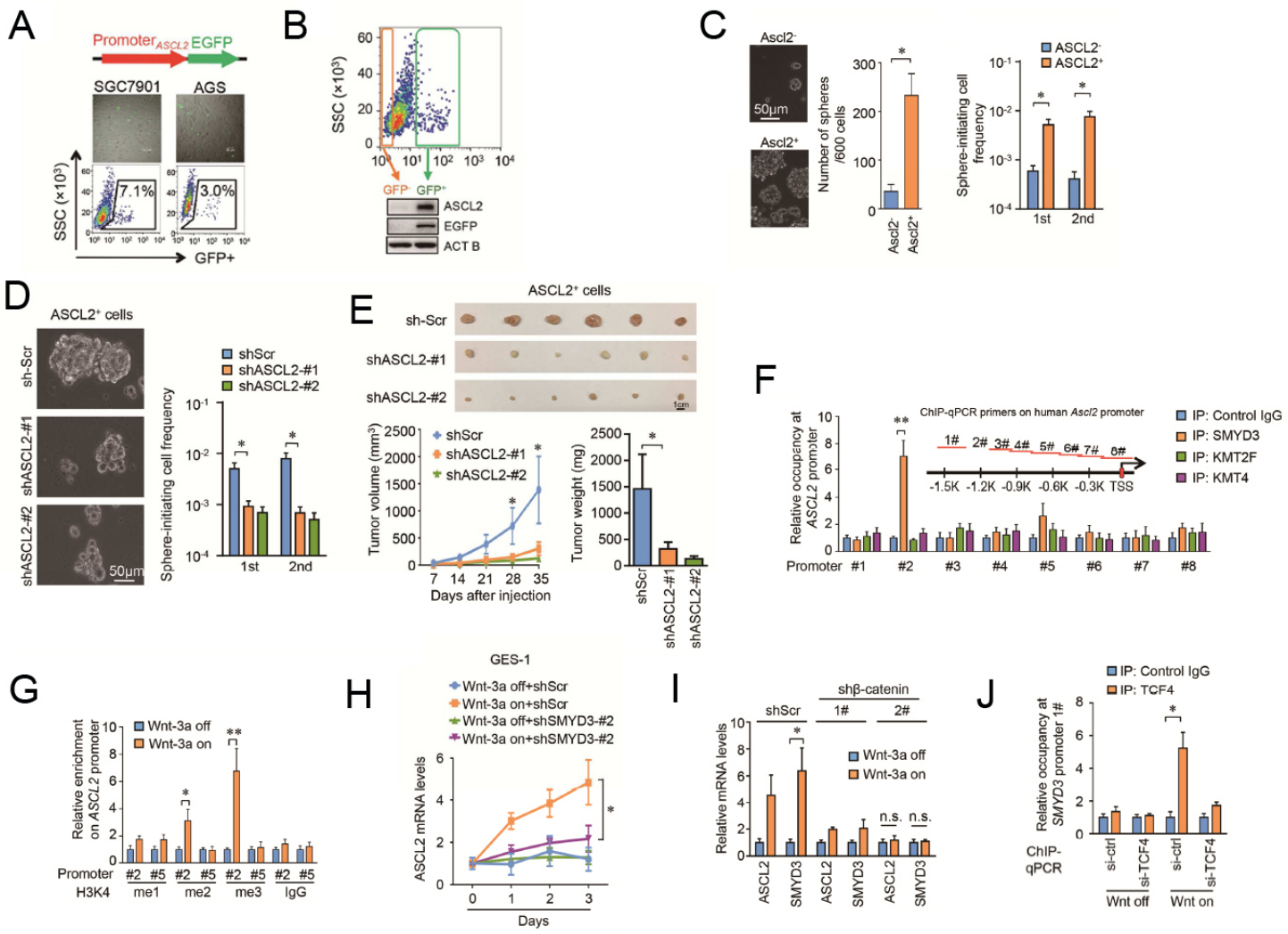
b. (4 points) You obtain tumor samples from patients that are part of a clinical trial to test the efficacy of hawkavir against an epithelial cancer. When you look at the histological sections of the tumors, you notice that patients that received hawkavir have substantially higher levels of dendritic cells, natural killer cells, and cytotoxic T cells than control patients.

How could hawkavir be inducing recruitment of these cells to the tumor? What experiment would you do to determine the role of each of these cells in hawkavir's oncolytic potential?

- Hawkavir enhances vascular leakiness
- Hawkavir induces the production of cytokines (eg IFNa/b) that recruit these cells to the tumor site.
- Determine hawkavir's oncolytic potential in mice deficient for DC, NK, or CTLs. Important to determine if viral replication and infection at tumor site is altered too.

Question 3: (Carlos Moreno)

Below is a figure adapted from a recent paper on gastric cancer:



The *Achaete-scute homolog 2* (ASCL2) gene is highly expressed in gastric stem cells and in gastric carcinoma (GC) cells. Although it is known that Wnt-3a can induce ASCL2 mRNA, previous ChIP-seq studies have shown that β-catenin does not localize to the ASCL2 promoter. The authors of this paper cloned the endogenous ASCL2 promoter upstream of a GFP reporter gene and stably infected a GC cell line with this reporter as shown in Panel A. They then sorted the cells into GFP^+ and GFP^- cells which were shown to be $ASCL2^+$ and $ASCL2^-$ by western blot in Panel B.

a. (3 points) What conclusions can you draw about the function of ASCL2 from the data in Panels C, D, and E? Why are there two shRNAs used in Panels D and E?

Answer: $ASCL2^+$ cells are enriched for cancer stem cells that can form spheroids. The shRNA experiments show that ASCL2 is required for spheroid formation in vitro in Panel D and for in vivo tumor formation in panel E. Two shRNAs are used to control for possible off-target effects and demonstrate specificity for ASCL2 knockdown.

b. (2 points) KMT4, KMT2F, and SMYD3 are lysine methyltransferase enzymes. What can you conclude from the data in panels F and G about the chromatin changes at the ASCL2 promoter in response to Wnt-3a? What controls are included in these panels and why?

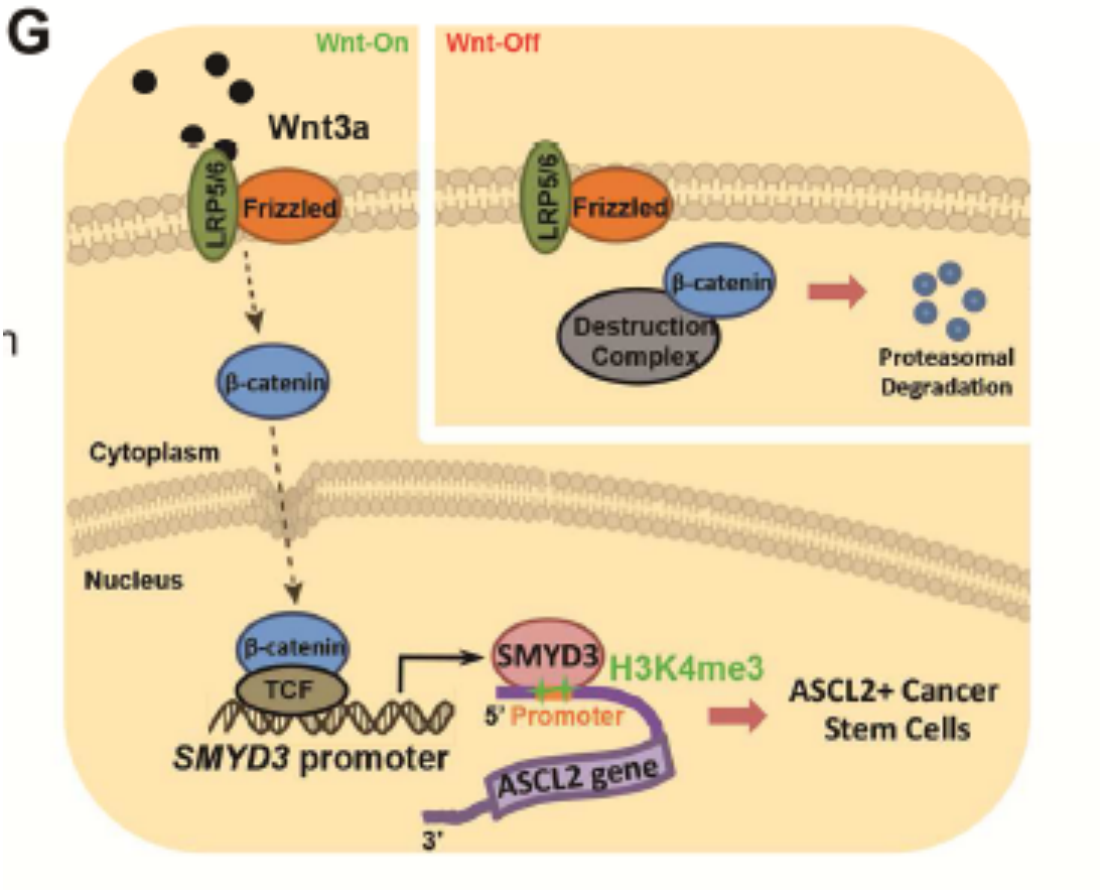
Answer: SMYD3 (but not KMT4 or KMT2F) localized to the region #2 upstream of the ASCL2 promoter in response to Wnt-3a and it catalyzes H3K4me3 at that locus. The IgG control is to monitor non-specific background from the ChIP pulldown. The #5 primers are used as a control for a region where SMYD3 is not bound.

c. (3 points) What can you conclude from Panels H, I, and J?

Answer: Wnt-3a induction of ASCL2 is dependent on SMYD3. Both SMYD3 and ASCL2 are dependent on β-catenin. TCF4 directly binds the SMYD3 promoter.

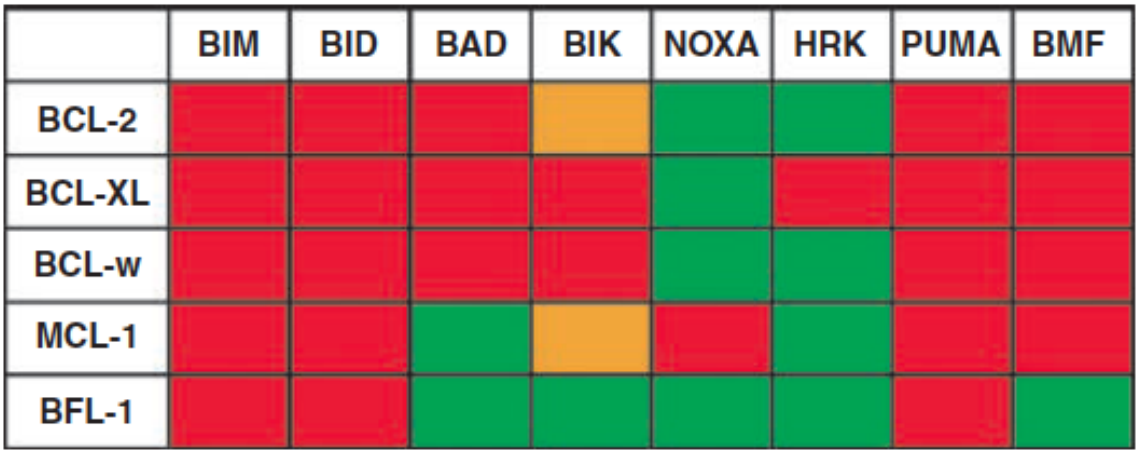
d. (2 points). Draw a model that summarizes the data from this figure.

Answer:



Question 4: (Larry Boise)

The diagram below illustrates the affinity of the 5 anti-apoptotic BCL2 proteins with 8 BH3-only BCL2 family members. Red indicates high affinity, orange is intermediate affinity and green is low affinity (not physiologically relevant).



a. (2 points) Based on what is presented here, which BH3-only proteins would you predict to be BH3-only activator proteins and which ones would be BH3-sensitizer proteins?

BIM, BID and PUMA are the activators. BAD, BIK, NOXA, BMF, and HRK are the sensitizers.

b. (3 points) Explain the rationale for why you assigned proteins as BH3-only activator proteins? As part of the explanation be sure to include the function of BH3-only activator proteins and how that guided your decision (diagrams are always acceptable as part of your answer).

Activators function by transiently binding to and activating the death effectors BAX and BAK. The anti-apoptotics function to neutralize the activators so they have to be able to bind to all three or the cell would be dead if any were expressed. The sensitizers function by inhibiting the anti-apoptotics to allow release of the activators so their binding is more fine-tuned to specific or at least selective interactions. They can't activate BAX or BAK on their own so they can't directly lead to killing, therefore they don't need to be neutralized like the activator proteins. Binding selectivity results in stress signaling only kills cell dependent on a single anti-apoptotic.

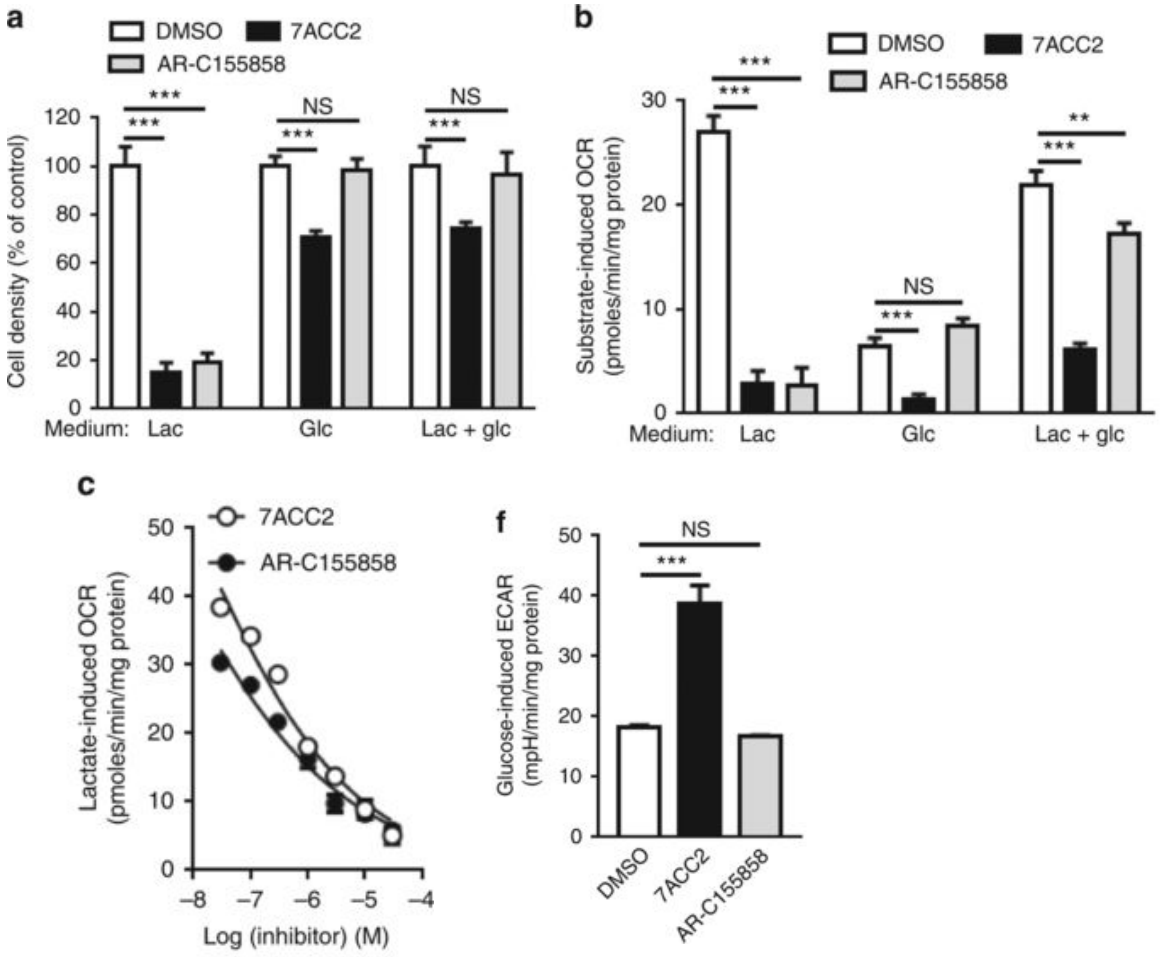
c. (3 points) If you were going to design a drug that mimicked the function of a BH3-only protein to selectively kill cancer cells, would you pick a BH3-only activator or sensitizer protein? Please explain why you picked one over the other and in your explanation be sure to include the molecular basis for why your drug would work to selectively kill cancer cells.

BH3-only sensitizer. An activator mimetic would kill any cell that has BAX and BAK and since normal tissues express these, the drug would be highly toxic. In contrast the sensitizer functions by binding to the anti-apoptotics to release activators. Therefore a sensitizer mimetic would only kill cells that are dependent on their anti-apoptotic proteins for survival. This is referred to as mitochondrial priming (occupied anti-apoptotics) and is much more common in cancer cells than normal cells (due to the stress response that is the result of oncogene-induced inappropriate cell proliferation). It is also the molecular basis for why cancer cells are more sensitive to most chemotherapeutic agents.

d. (2 points) Design an experiment that demonstrates that your drug is working by the mechanism that you predict. Be sure to include what your readout will be and how it will measure drug activity.

There are lots of possibilities here. The best answers would be a demonstration that the drug causes the dissociation of BH3 activator from an anti-apoptotic however one could use isolated mitochondria and demonstrate cytochrome c release from cancer cells but not normal ones. I suspect some students might just try a death assay. This is okay but does not directly demonstrate mechanism, lots of stuff you throw on a cell will kill it or cause cyto c release but that could be due to a mechanism upstream of inhibiting BCL2.

Question 5: (Mala Shanmugan)



One broadly applicable characteristic of cancer cells is their increased avidity for glucose and preferential use of aerobic glycolysis that has come to be known as the Warburg effect. Glycolysis produces pyruvate, which can be reduced to lactate or oxidized to acetyl-CoA to support the TCA cycle and oxidative phosphorylation (OXPHOS) in the mitochondria.

Lactate itself, however, can also serve as a major carbon source to sustain the TCA cycle and OXPHOS, as lactate imported into the cell can be oxidized to pyruvate, freeing up glucose for other cellular functions. As a result, inhibitors that target glucose metabolism *or* lactate import alone are rather ineffective as single agents for inhibiting cancer cell growth.

Lactate import across the plasma membrane is primarily mediated via the monocarboxylate transporter MCT-1, whereas pyruvate import to the mitochondria is mediated by the mitochondrial pyruvate carrier (MPC). The inhibitors **AR-C155858** and **7ACC2** reduce lactate import significantly (data not shown). Recent investigations with these two agents have shown that they also impact proliferation (panel A) and oxygen consumption rate (OCR) (Panel B) when cancer cells are cultured in media containing lactate alone (no glucose). However, when cells are cultured in media containing either glucose alone or both glucose and lactate, only inhibitor **7ACC2** reduces cell proliferation (Panel A) and OCR significantly (Panel B).

Although both inhibitors reduce lactate-sustained OCR (Panel C) only **7ACC2** increases the extracellular acidification rate (ECAR) of cells cultured in glucose (Panel F). The investigators

hypothesize that **7ACC2** inhibits the mitochondrial pyruvate carrier (MPC) and that one of the effects is to inhibit lactate import.

a. (2 points) Which two panels/data above suggest that these cells rely on lactate import to sustain oxidative phosphorylation? Please provide a rationale for how the experiments support this conclusion.

Panels B and C, in B when you culture with lactate alone OCR is maintained but blocked with both inhibitors, when cultured in glucose alone OCR goes down. Panel C shows that the inhibitors block OCR sustained by lactate.

b. (2 points) Which data/panels above suggest that the effects of **7ACC2** are indirect (i.e., not via direct binding of the drug to MCT-1) and likely on the mitochondrial pyruvate carrier and why? (2 points)

Panels A, B and F. **7ACC2** inhibits proliferation and OCR as seen in panel A and B respectively, in cells cultured in glucose or lactate or both, and **7A** inhibits OCR in cells cultured with glucose alone, also **7ACC2** increases glucose induced ECAR (because the utilization of glucose in OXPHOS is blocked with the accumulated pyruvate)

c. (2 points) Which inhibitor would you prefer to use to treat this cancer and why?

7ACC2 because it blocks lactate import and pyruvate oxidation.

d. (2 points) How would you test whether **7ACC2** targets the mitochondrial pyruvate carrier MPC, and not MCT-1?

Demonstrate pyruvate accumulation, KD the MPC or MCT1 transporters and show lack of efficacy with the MPC KD and pyruvate accumulation in the MCT1 KD.

e. (2 points) If you were able to only administer the **AR-C155858** compound which other metabolic pathway would you target to elicit cytotoxic effects.

Target compensatory OXPHOS with mitochondrial TCA/OXPHOS inhibitors.

Question 6: (Renee Read)

You've started as a new PI at Winship. Your just isolated a new gene, named *tenure project*, that, based on genetic assays, regulates signaling downstream of the EGFR receptor tyrosine kinase in gut development, and, in your newly awarded NIH grant, you've proposed to determine the function of *tenure project* (TP) in colon tumor cells.

a. (3 points). Name three domains that you would immediately look for in the protein sequence of TP. (Note that there are more than three possible domains of interest but you should pick three and only three for your answer). Describe the functions of these domains.

- SH2 domain – binds to phosphotyrosine
- PTB domain – binds to phosphotyrosine
- SH3 domain – binds to polyproline-rich regions
- GTPase domain – hydrolysizes GTP to GDP
- GAP domain – accelerates GTP hydrolysis by GTPase domain
- Tyrosine kinase domain – uses ATP to phosphorylate tyrosine residues on substrates
- Serine/threonine kinase domain – uses ATP to phosphorylate threonine and serine residues on substrates
- Phosphatase domain – hydrolysis phospho-amino acids (tyrosine, serine, threonine) on proteins

b. (4 points) You transfect EGFR-negative colon cancer cells with EGFR and TP, and discover that, in response to EGF treatment, TP binds to EGFR in co-immunoprecipitation assays. What does this result indicate about the interaction between TP and EGFR? Draw a model showing the mechanism of TP binding to EGFR. Design an experiment using colon cancer cells to test your model of the mechanism of TP binding to EGFR.

- TP binds to activated and phosphorylated EGFR at a phospho-tyrosine residue in the cytoplasmic region of EGFR. To test this, you could create two mutant variants of EGFR, a catalytically dead mutant (EGFR-KD) and a mutant in which tyrosine residues are replaced with phenylalanine residues (EGFR-YF) such that no tyrosine autophosphorylation would occur. Control wild-type EGFR (EGFR-WT), EGFR-KD, and EGFR-YF could be expressed in colon cancer cells, and these cells could be cultured as follows: EGFR-WT, EGFR-KD, and EGFR-YF cells with EGF and EGFR-WT, EGFR-KD, and EGFR-YF cells with ligand. EGFR could be immunoprecipipated from all of these cells, and the IPs could be run on a western blot that is then probed with TP. If TP binds to phospho-tyrosine residues in an activity-dependent manner, then TP should fail to bind to EGFR is all cells not treated with ligand, and fail to bind to EGFR-KD and EGFR-YF from EGF treated cells.

- Another option is to treat cells with an EGFR kinase inhibitor to determine if TP binding to EGFR+EGF is reduced when EGFR catalytic activity is blocked. An important control for the inhibitor experiment will be to confirm loss of autophosphorylation in EGFR treated with inhibitors.

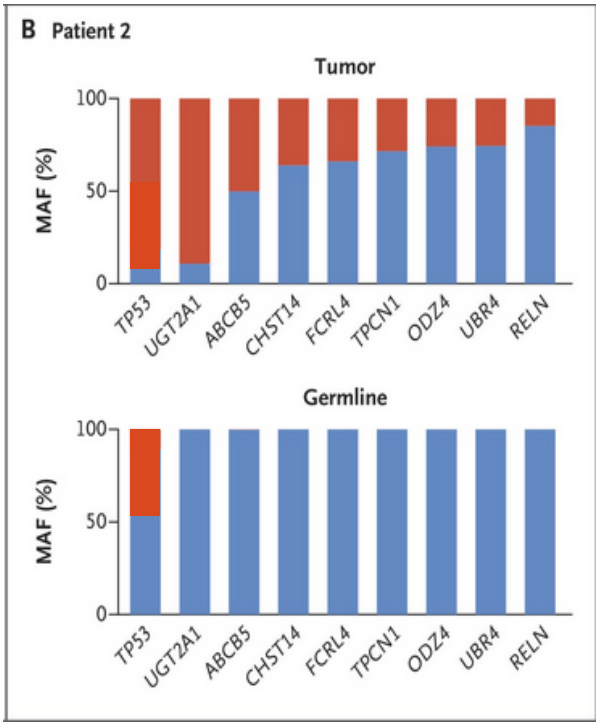
c. (3 points) You do a second experiment to determine if TP binds to GTP-loaded Ras or GDP-loaded Ras. What is the difference between these two forms of Ras and how are they related to EGFR signaling? You find that TP only binds to GTP-loaded Ras. What would you conclude about TP's interactions with Ras and why?

- GTP-Ras is activated and stimulates the membrane recruitment and catalytic activation of Raf and the activation of the MEK-MAP kinase pathway. GDP-Ras is the inactive version. Therefore, TP only binds to activated GTP-bound Ras. TP may either contain a GAP domain, and bind to Ras to stimulate GTP hydrolysis and thereby down regulate Ras signaling, or TP

may be a positive effector of Ras signaling, and be recruited to the membrane to be activated by binding to GTP-bound Ras, like Raf or PI-3 kinase.

Question 7: (Paula Vertino)

Recent findings from the Pediatric Cancer Genome Project (588 children; median age 6.9 years) and a similar genome project of pediatric cancers in Germany found that ~8% of children and young adults with cancer carry a germline predisposing mutation. Unlike adult tumors, less than half of these cases showed any evidence of a family history of cancer. Data were derived by exome sequencing or whole genome sequencing of PBMCs (peripheral blood mononuclear cells) or buccal cells (cheek mucosa) and tumor tissue from the same patient. Results for a subset of genes in a single patient are shown below:



MAF= allele frequency

a. (2 points) What is your interpretation for how the genes differ ie. if you had to classify them as potential oncogenes or tumor suppressor genes, how would you do so? Justify your answer.

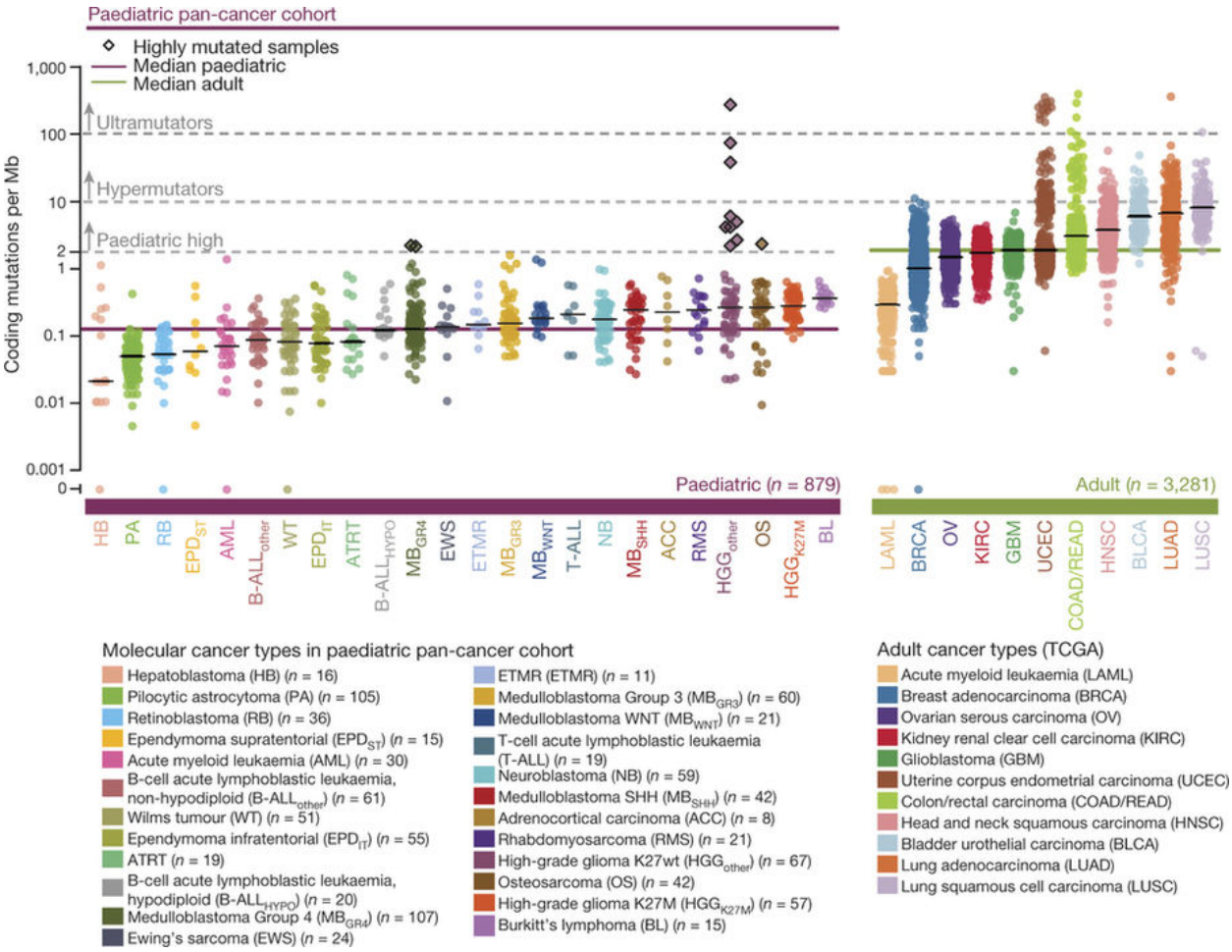
- TP53 and UGT2A1 are TSG; the rest as oncogenes. The finding that there is 100% mutant in tumor suggests that p53/UGT2A1 are TSG (eg. biallelic loss of function) and the rest at 50% or less (ie. one mutant allele) likely oncogenes

b. (2 points) With regards to patient 2, what is your interpretation of the differences observed between p53 (TP53) and UGT2A? As part of your answer explain the relationship between the germline data and the tumor data for each gene and a likely scenario for how each pattern arose. What critical concept in cancer genetic do these two examples illustrate?

- TP53 exhibits the inheritance of one mutant allele observable in the germline, in the tumor the wt allele is lost so only mutant is observed, thus this is an example of inherited or germline predisposition or a familial cancer syndrome (Li-Fraumeni). UGT2A1 also exhibits biallelic loss in the tumor (eg. only mutant allele exists the other likely lost to LOH), but you see only wt in the germline, therefore the two 'hits' occur somatically. Knudsen's hypothesis.

c. (2 points) The Figure below shows the burden of somatic mutations among pediatric tumors (left side) relative to adult tumors (right side). What does the overall pattern suggest with regards to total mutation burden between the two and why is this the case?. If you plotted the same data within a particular tumor type how would you expect the data to stratify? …that is, within each pediatric tumor type the mutational burden is distributed across 1-2 orders of magnitude….what might account for this variability from low to high? (draw a graph if its easier)

As expected there is a lower mutational burden among pediatric cancers than adult cancers based on age and the "somatic mutation theory" eg. relationship between mutation accumulation and age. If one takes one tumor type, the dots likely stratify by age with the oldest child having the greatest burden.



d. (3 points) In spite of the above, certain pediatric tumor types appear to have just as high a burden as some adult tumors, including a subset of High Grade Gliomas and Group 4 Medulloblastomas.

Noting the similarity to some adult cancers, provide an explanation for what might account for the hogh mutational burden. How would you test your hypothesis given all the genomic information you have at hand for this cohort?

Provide one potential explanation for the difference between "ultra" high mutator HGGs and the regular hypermutators, keeping in mind that these tumor arise within the first 2 years of life typically.

- These hypermutators have a mutation burden that rivals that of endometrial cancer and colon cancer, ie. those driven by alterations in mismatch repair. Given all the exome sequencing data available one could look mutations in these genes (MSH2 MSH6 PMS2) and their relationship to

mutation burden. Both the germline and tumor should be examined. All were associated with germline mutations in MSH6, MSH2 or PMS2. The "Ultra" high mutators were associated with biallelic germline inactivation. (While adult lung and bladder have a similar mutation burden, these are likely the result of carcinogen exposure…given that these are pediatric tumors this is unlikely to be a significant factor in these cases)

e. (1 point) While not currently used in this population, immune checkpoint therapy is now being considered for some pediatric cancers based on these data. Given your understanding of immune checkpoint therapy, which pediatric tumors are likely to have a favorable response and why?

- Hyper/ultra mutators…High grade gliomas w/o K27M mutations, maybe group 4 Medulloblasstomas. A good response to immune checkpoint therapy is thought to stem from the neo-antigens presented on the tumor. The high rate of mutation in these tumors is more likely to generate such neo-antigens.

Question 8: (Melissa Gilbert-Ross)

A genetic mosaic screen was performed in a model organism to identify genes that restrict tissue growth. The goal was to identify novel conserved tumor suppressor genes. One of the genes that emerged from the screen was *sautéed potato-eye* (*stp),* which has four well-conserved human homologs. You are interested in in determining whether these homologs have a role in mammalian tumorigenesis.

a. (3 points) Prior to starting experiments, what bioinformatic approaches can you use to determine whether any of the four human homologs might be tumor suppressors in human cancer?

- Bioinformatic analyses using public cancer databases to investigate somatic mutations/copy number/protein expression for the human homologs (COSMIC/ cBioPortal/Protein Atlas)…. loss of function would imply TSG….LOH, deletion, truncating mutations, etc To take it one step further you can use these databases to test whether the loss of STP family member expression correlates with reduced survival… (PV comment: relationship to survival does not necessarily imply a TSG or oncogenic (driver) function, and the expression of many known onc/tsg are not predictive of outcome)

b. (2 points) Based on your answer to part a, above, you find evidence that one of the four homologs, STP2, may in fact be a tumor suppressor gene. A colleague offers you an STP2-deficient lung cancer cell line that forms tumors when injected into immunodeficient mice. Design an experiment to investigate whether loss of STP2 function indeed contributes to the tumorigenic phenotype in this line

- Overexpress the wild type *STP2* gene in the lung cancer cell line and test it in the xenograft assay.

c. (3 points) Again based on your investigation in part a, above, you hypothesize that loss of a single copy of *STP2* in the germline *(*haploinsufficiency*)* would predispose to lung cancer. Design an experiment to test your hypothesis using a genetically engineered mouse model.

- You generate a genomic loss-of-function allele of the moiuse ortholog (*Stp2)* \ and perform an intercross with heterozygotes. You age and test wild-type (Stp2 +/+), heterozygous (Stp2 +/-) and homozygous (Stp2 -/-) progeny for tumor phenotypes.

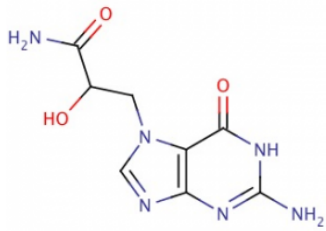
d. (2 points). While carrying out your experiment in part c, above you discover that homozygous loss of *Stp2* is lethal. Moreover, you have extended your analysis in part a and discovered that LOH of *STP2* commonly co-occurs with oncogenic KRAS mutations in lung cancer. You hypothesize that loss of *STP2* promotes the progression of KRAS-mutant lung cancers. Design an experiment to test your hypothesis in a genetically engineered mouse model.

- You generate a conditional floxed allele of *Stp2* in the mouse and cross this to a mouse carrying a conditionally activatable (loxP-STOP-loxP) Kras allele and induce lung cancer using a tissue/cell-type specific Cre transgene or by delivering viral-Cre to the lungs. Possible phenotypes to measure are tumor burden, tumor grade, tumor multiplicity, early invasion, dissemination to lymph nodes and metastasis to distant organs in both experimental (activated Kras $^{G12D/G12V/G12C}$ and deleted Sfv) and control mice (activated Kras alone).

Question 9 (Bill Dynan)

California proposition 65, passed in 1986, mandates that businesses place a warning label on products containing any of 1065 chemicals that the present risks of “cancer, birth defects or other reproductive harm.” Proposition 65 has been in the news recently because a California judge ruled that Starbuck’s coffee must carry a warning label based on the presence of trace amounts of acrylamide, which is formed during the roasting process.

The mechanism of acrylamide carcinogenesis involves metabolic conversion to glycidamide, which forms DNA adducts including N7(2-carbamoyl-2-hydroxyethyl) guanine, (N7-GA-Gua), A structure is below. This adduct is present in the DNA of animals exposed to high doses of acrylamide. It is also known to be released from adducted DNA, as a free base, by a repair process.



a. (2 points). Briefly describe experimental approaches that might have been used to determine (i) that acrylamide is carcinogenic, and (ii) that the metabolite, glycidamide, and not acrylamide itself is the ultimate carcinogen form.

- Administer to animals in some form and look for tumors. Also acceptable, in vitro cell transformation assays.
- Glycidamide might be observed as a metabolite; glycidamide but not acrylamide is active in a mammalian or bacterial-bsed assay for transformation or mutagenesis (for example the Ames test).

b. (4 points) Assume that base excision repair is responsible for repair of N7-GA-Gua modified DNA. List the sequential steps in base excision repair, including the step that releases the free base.

- Removal of damaged base by DNA glycosidase (releases free base)
- Removal of sugar residue by apurinic endonuclease (APE1)
- Fill-in of gap by DNA polymerase
- Strand rejoining by DNA ligase

b. (2 points) In a cell culture model, what are the likely consequences of failure to repair the N7-GA-Gua lesion?

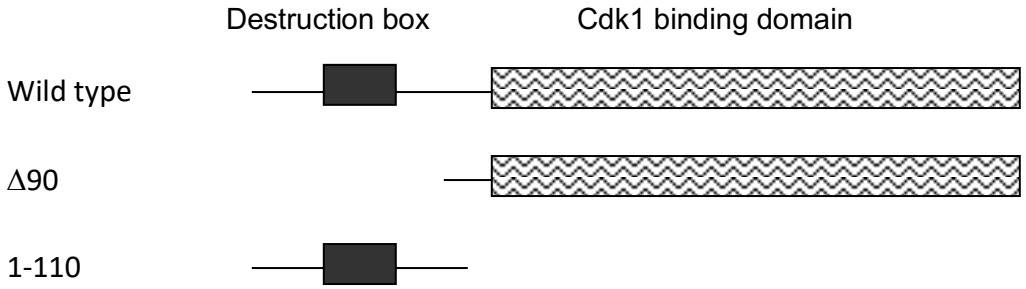
- Mutation as the result of translesion synthesis, replication fork stalling/collapse, death

c. (2 points) Design an experiment in cultured fibroblasts to determine whether base excision repair is indeed the mechanism for repair of N7-GA-Gua lesions.

- Knock down specific DNA glyscosidases, or APE1, to see if this interferes with repair (adduct not released, or toxicity/mutagenicity/cell transformation increased)
- Alternatively use knockout mouse fibroblasts in same assays.

Question 10 (Maureen Powers)

While studying the requirements for sister chromatid separation in anaphase, you separately overexpress each of the following mutant versions of cyclin B in cells. Cyclin BΔ90 is missing the destruction box required for recognition by the APC. Cyclin B 1-110 retains the destruction box but cannot bind to Cdk1.



When either mutant cyclin is expressed, MPF activity remains high and cells do not exit mitosis. The two mutant proteins differ in the stage at which transfected cells arrest. When cyclin BΔ90 is overexpressed, sister chromatids separate normally and cells arrest in anaphase. When cyclin B 1-110 is overexpressed, sister chromatids do not separate and cells arrest in metaphase.

a. (2 points) Why does MPF activity remain high in the presence of cyclinBΔ90?

- Excess Cdk1 activation domain that cannot be destroyed by the APC. Even when the APC is activated the mutant cyclin cannot be degraded and continues to activate the endogenous Cdk1.

b. (2 points) Why does MPF activity remain high in the presence of cyclinB1-110?

- This form provides an excess of substrate for the APC and thus competes for the endogenous cyclin. The result is that endogenous cyclin is not degraded and continues to bind and activate cdk1.

c. (2 points) What does this experiment tell you about the respective contributions of cyclinB and the APC to sister chromatid separation?

- APC function and thus ubiquitin mediated protein degradation is necessary for sister chromatid separation but the degradation specifically of cyclin is not required for sister chromatid separation.

d. (4 points) In cells with the following mutations, would the cell cycle be arrested, yes or no. If yes, at what stage of the cycle would arrest occur and why?

i. non-degradable mitotic cyclin

- Anaphase arrest, can't complete sister chromatid separation
- (half credit for metaphase arrest)

ii. overexpression of p21

- G1 arrest Can't activate S phase cyclin/cdk

iii. non-phosphorylatable Rb

- G1 arrest Can't express S phase factors

v. constituitive E2F expression

- Cells do not arrest, they continue to cycle