Aaron: Clonal evolution + genome evolution

Samples

- PE from Leila last week
  - *Shipped o/n 4C*
  - *Chunk of metastatic tissue from lung*
    - Minced, cryostore
  - *Cell pellet*
  - *Plasma/liquid*
- Extracted DNA and RNA from tissue
- Cell pellet: RNA
- All stored -80
- Saliva from Tesselo: look for virus if he also carries it

Help from steve

Subclonal

Variant allele freqs – CeGaT data

- x-axis:normal freq (# reads aligned with alternate base)
  - *if all alt, 1, if half alt, 50%*
  - *expect max 50% b/c 2 alleles normal*
- y-axis tumor allele freq
  - *most private to tumor*
  - *expected with somatic variants*
  - *a few seen in both normal + tumor*
  - *(line shows same level in both) – above = more present in tumor*
- Off-axis genes labeled
  - *Known Cancer drivers labeled*
  - *Filterd for depth, selected for things called as somatic vars*
- CREBBP showed up higher in y-axis --- more in tumor tissue
- Algo to cluster
  - *MCLUST*
  - *Mixture models and normal dist's*
  - *K=4: subclonal, major clonal group*
  - *Main mut in clonal group: CREBBP*
- Note: this is from first tumor, much has probably chaged and been selected for
- MLH1 interesting to Dr Castro
  - *Related to DNA-repair, so maybe included in MSK panel, but not called?*
    - Likely variant calling filtered out, maybe too much in normal
- subclonal and clonal list to be put on slack
- threshold by reads, heuristically, somewhere ~20 reads

next steps

- manual check to verify calls

look at RNA profile
alignment with pathways Castro mentioned
are any known to be strongly selected for?
actual DNDS score?

Q: concern with germline mutations and effects on tumor and predisposition?

Q: overlap with regulatory regions? Exome makes it hard; being epigenetically driven cancer, that would be very helpful to have

Cosmo/Onno – Virus hunters

Unmapped reads: 33K

NCBI automated tool to tell what other orgs found in seq data

Onno: found AI-based solution as someones phd thesis

ViraMiner
Pre-trained >> check how trained? Traina agin ourselves?

Done on both tumor and normal

Control: all mapped reads and check those

Ranked reads based on score

Tools; google collab

Tumor tissue unmapped

11% viral
With prob cutoff score 0.5 (a bit arb)

Q: change cutoff to higher, see more diff between normal/tumor

Normal unmapped: 9.5% (not significantly lower)

tumor mapped: 0.7%

Prob not trained on HERV seqs
Could also do on bacteria, viral, illumina noise, to see different sources

Find virus on BLAST

It is a certain virus

Next steps

Map to suggested virus(es), score viruses
Freq of viruses
Run on other data (RNA, WGS)
Train on HERV
Compare to other human genomes
Esp b/c should be similar across people, if HERVs really so dead

Q: Xiaowei: HERVs very fixed in genome, can be transcribed, RNA can have effects, but not expect DNA amplification

Q: species you see? Mostly phage

Q: contamination or from tissue? A: prob contam, esp b/c of external nature of tissue

Q: how do you know no integration possible? A: some can still produce proteins, even viral-like; virtually all defective in some way, cannot jump

back to host; in mouse, though, still active; primates fixed for millions yrs

Only one study ever found one jumping back, very much doubted

Q: differentially mapped—any specific class? A: not check types of viruses

Xiaowei: CeGaT data reanalysis

Big Q: hat are differences b/t tumor and control

Somatic snvs

Saw CREBBP

Redid, focused at lower freq point muts, not reported

Since earlier time point, maybe still at lower freq, higher now

11 point muts in cancer driver genes

1.8-4.8% cells for 9/11

TNFAIP3-16%

CREBBP-38.6%

*see slide w/ chart

Unsure if these are LOF or GOF

With new sample, see how freqs change, some are more dominant and can be targeted?

COSMO: PIK3R1 mut in SH2 domain would be big news; BUT low freq so ???

Checked ALL somatic muts, highest still ~40%, so looks like a very heterogenous tumor in general

Maybe low freqs are due to normal tissue contam??

**PATHWAY ANALYSIS OF SOMATIC MUTS – meenaskhi + flor (see slide)

Q: number of genes after filtering? A: ???

Reactome pathways: high p-vals, no clear enrichment

Drug prediction: Valproic acid

Most significant, most genes

ECGC also shon up (top on graph)

Digoxigenin also mentioned by Castro

May imply these drugs can go after multiple subclones?

Germline snvs

In known cancer drivers: may be predispoisition muts

Can these be targetable?

Need to check to make sure these are true; also homozygous/hetero

Looks like a lot… hmmm….

Cannot speak to how much this really is, lot/little

Retrotranspositions

3 still active in humans: Alu, L1, SVA

No relevant L1 or Alu insertions

RNA-seq would help see: they are polyA'ed

- Structural variations – Han
  - Both CeGaT and MSK
  - Confirmed SMARCB1 deletion
  - Confirmed MAP3K14 deletion
  - NEW amplification of RAP2B
    - *Looks like ~1 additional copy*
    - *Not targeted by MSK, by chance shows up here*
    - ***risky to call true CNVs from exon*
    - *New data (WGS) would help (10-20x)*
- Loss of heterozygosity
  - 'balance' from backup allele can be deleted in cancers
  - No sig LOH for cancer driver genes
  - For all germline muts found in tumor and normal, is there change in freq? (going from het in normal to homozygous in tumor)
  - 3 SNPs found
    - *FMN2 – 0.29>0.99*
    - *TCAF2 - 0.47> 1.0*
    - *ITGAL – 0.44>0.71*
- Chromosome aneuploidy
  - Signature for highly metastatic
  - Gain/loss of whole chrom
  - No diff b/t normal and tumor >> no large scale aneuploidy
  - Should check for other tissues
- Future
  - New samples: did any of low-freq clones become dom? i.e. radiation resistant
  - Integrative analysis w/ other exps
    - *Transcriptome, methylome, ATAC*
    - *Validate findings for CN*
  - Anti-HERV antibodies available – can check cells for expression by stain
    - *Concern: epitope being presented may not be the exact one that gets tested for – the viral mimicry driven by dsRNA, not envelope (which this antibody detects)*
      - Lesson: wouldn't exclude
      - Fix: qpcr on multiple domains of HERV

Monica: Cell cycle implications of EZH2

- Transcriptomics
- Previously work on endothelial cells, when EZH2 is disturbed
  - Her work: EZH2 knock down, leila's tumor: EZH2 overactive
- Regulates cell cycle related genes (KO makes low cell cycle genes)
  - So up EZH2 >> up cell cycle possible
- Very strongly connected gene network > dozens
- Cyclins downregulated with EZH2 KO

- Also proliferation
- BUT remember EZH2 is methyltransferase in polycomb
  - Adds trimethyl on H3K4
  - Thought: EZH2 inhibits something which inhibits cell cycle driving genes
    - *Represses the repressor*
  - Big Q: what is upstream of all these cell cycle genes?
    - *Possible: TXNIP*
      - Tumor suppressor, upreg inhibits cancer growth
- Q; Taz is EZH2 inhibitor, makes sense
- Q: druggable targets in list of EZH2-reg genes?
  - MAPK upstream may be targeted already
  - Future: this list of most affected EZH2 genes (in endothelial cells) may be affected in ES

Future Wet lab: Karen + Saraubh

- Immediate clinical pathway
  - **find another actionable path: AIM only 2x more**
  - Previous samples:
    - *Diagnostic slide: used to say 'yes this is ES'*
      - Not used to assess lymphocyte
      - Need to know if tumor is immunoinfiltrated for immunotherapy
      - Ben + Ariel have friend Kelly to look
  - Current sample
    - *Pleural effusion*
    - *Tumor?*
      - Yes: do we have enough sample?
        - Qpcr, Rna
        - Immune infiltration
        - HERVK expression: implications for immunotherapy
        - ***are there actionable variants?
      - No:
        - Blood
        - PET scan

- Current tissue analysis
  - Cofirm if tumor, what %
    - *"SMARC Aleck" PCR test*
      - Flanking region primers on SMARCB1
    - *Look at PE sample under H&E stain, pathologist*
    - ***important to determine next steps*
    - *Computationally deconvolve cell types (cybersort)*

- *Quantify by flow cytometry or microscopy*
- What genes/pathways dysregulated? Controls?
  - *Comparative analysis: GTEX (normal tissues) and TCGA (cancer tissues) for comparison*
    - Histogram litmus – compare patent pathway expression to distribution from all control tissues (from GTEX) and from all cancer tissues (TCGA)
  - *Sant Chawla confirmed blood or skin ~ comparator tissue*

Future sample procurement and testing

- Transcriptome analysis
- Blood
  - *Whole exome: ctDNA (CeGaT) for heterogeneity*
  - *Test for ascorbate, zinc, vit d, taurine*
  - *Test for Abs – HERVK*
  - *"normal tissue" for RNA-seq*
  - *Procurement*
    - STREK tubes in cold pack o/n to Germany
    - STREK tubes in cold pack o/n to Natera
- Fresh tissue
  - *WGS*
  - *RNA-seq*
  - *Spheroid/organoid (Sengine)*
  - *Procurement:*
    - CT to find accessible tumor
- Pleural fluid
  - *Organoids*
- Stool
  - *Viome.com*
  - *Are pops there that are necessary for immunotherapy*
  - *Repeat in weeks, see if improvement*

Prioritization of new wetlab experiments

- See spreadsheet
- Most important: tumor content of PE tissue sample

MSK Red Tape Busters

Problem: dangerous to keep Leila with current medical team

- Unwilling to take risks
- Absentee

Solution: change care team

- Discuss changing docs with MSK brass (dr. tap > boss of gounder)
  - *Assess willingness of another doc at MSK to act of consensus recs*
- If no go, change hospitals ASAP

Tassilo already in touch with two other NYC-based physicians with rep for responsible risk appetite

Fereshteh analysis

- Variant analysis
- Try to find some variants that explain invasiveness
  - Very hard to find
- Would benefit from reseq what we have, put value on germline mutations
- New sample: WES, WGS
- No family background in cancer in this case
  - Could be de novo
  - Could be totally due to env't
    - *Egg removal, what was the decision here?*
    - *Very rare cases of metastatic ovarian cancer to genitals or lymh node*
      - -angiosarcoma(?)
- Future: maybe CLIA metabolic analysis
  - Some insight about what to push or avoid
- Natural remedies
  - Crude poppy extract, eaten
  - Apoptotic agent
  - Similar to heroine/morphine(?)