Burkitt Tumor RNA seq

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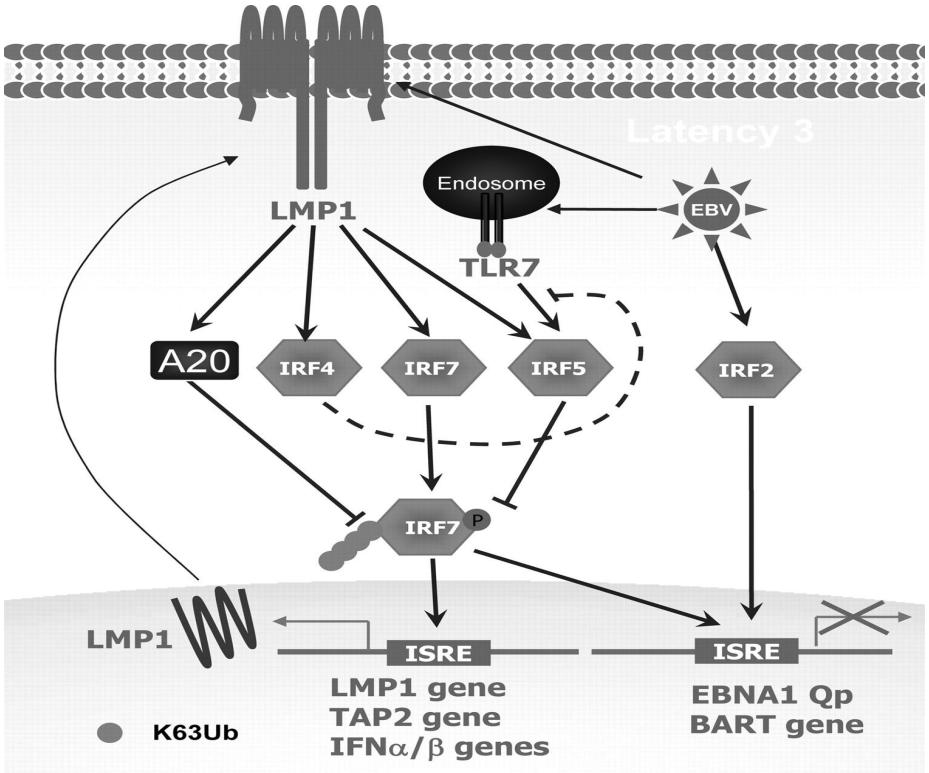
Initial question

What can we learn from tumor biopsy RNA sequencing data?

LMP1



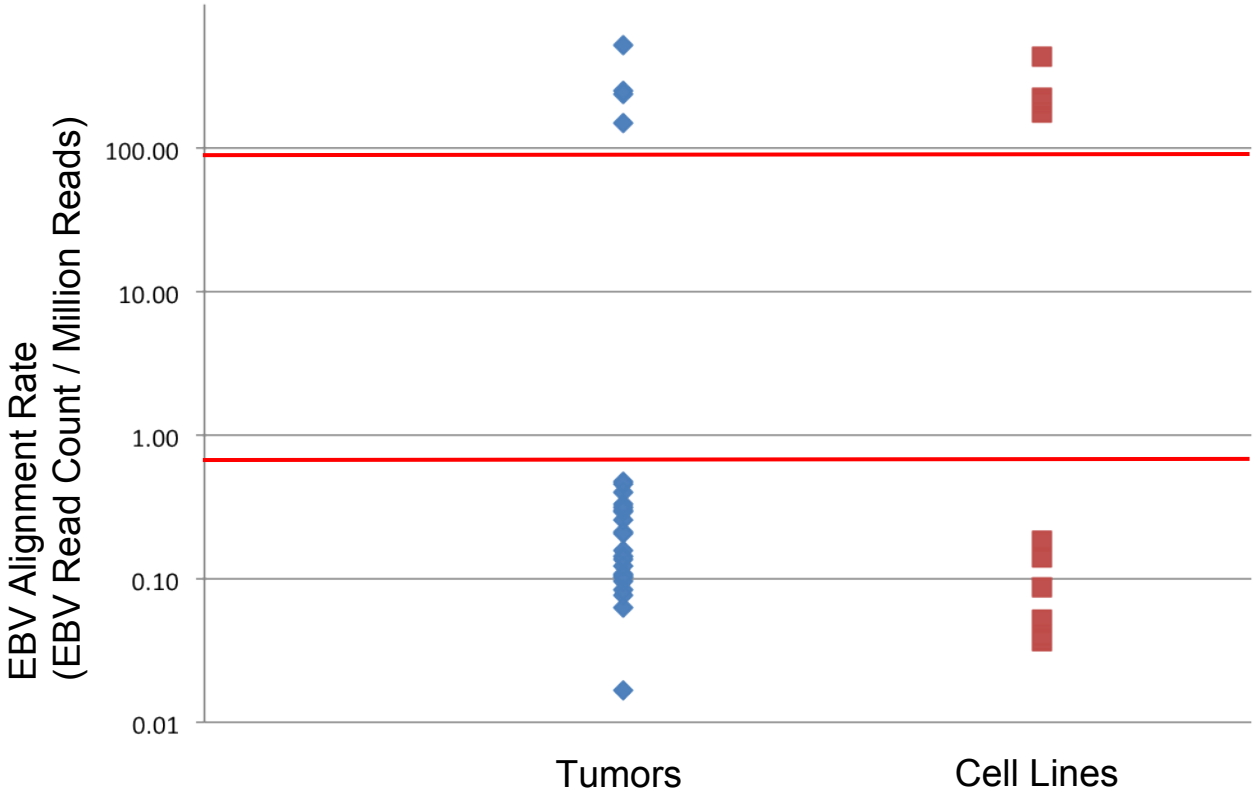
- We know that LMP1 affects host gene expression
- We also know that host transcription factors affect LMP1 expression
- We can use the RNA sequence data to quantify both host gene expression and EBV gene expression
- Therefore we can search for other ‘circular’ interactions like LMP1
- And compare actual tumors to our model cell lines

Ning S, Pagano JS. The A20 Deubiquitinase Activity Negatively Regulates LMP1 Activation of IRF7 . *Journal of Virology*. 2010;84(12):6130-6138.

Overview

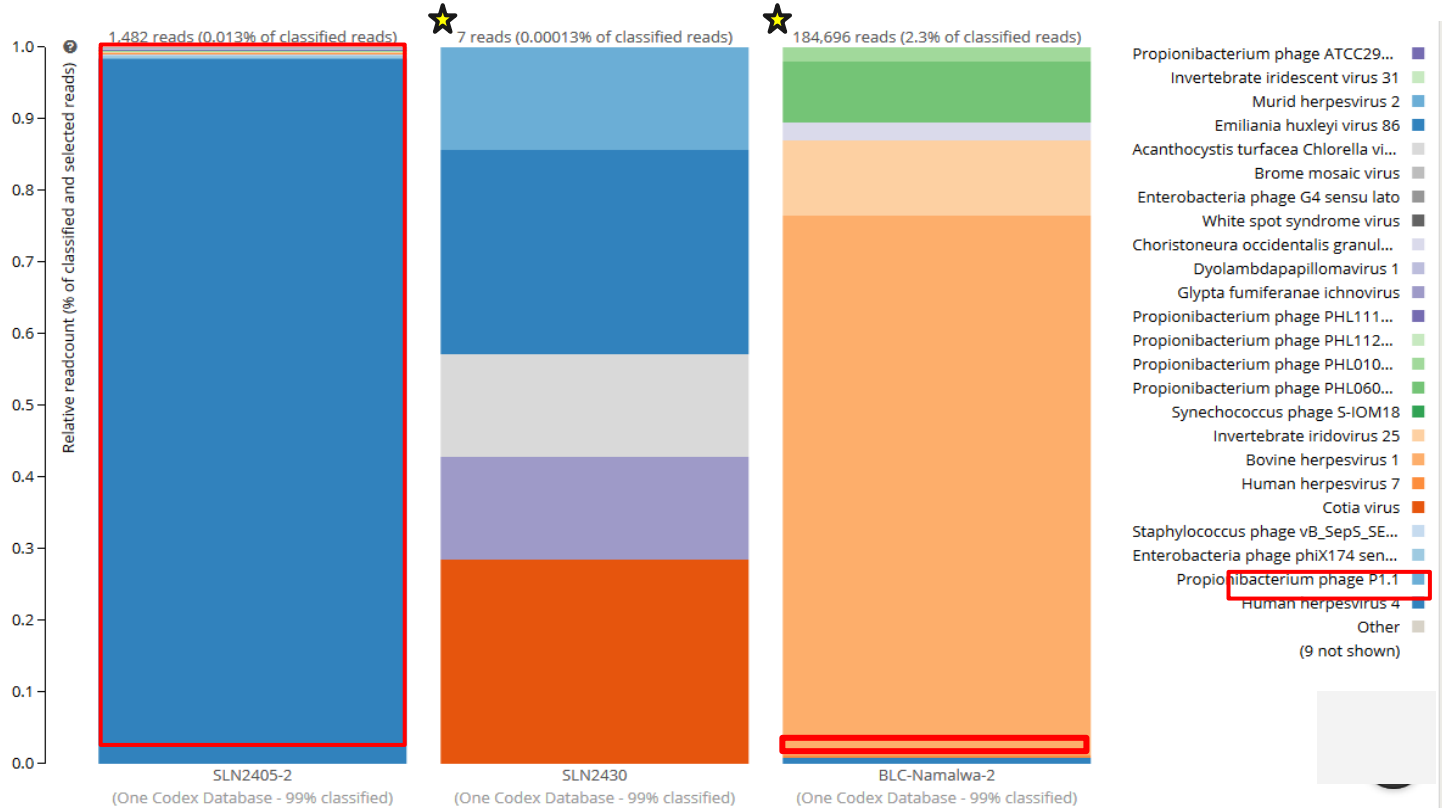
- Acquire RNA sequencing data from SRA
  - 28 Burkitt Lymphoma tumors
  - 5 cell lines
- Determine EBV positivity of samples
  - Also quantify EBV gene expression
  - Compare EBV+ tumors to EBV+ cell lines
- Perform host gene differential expression
- Attempt to tie together host differences and EBV differences
- Make comparisons between tumor and cell line

Determining EBV positivity



- EBV alignment rate follows a binomial distribution
- We can classify the high group as positive
- And the low group as negative

Determining EBV positivity

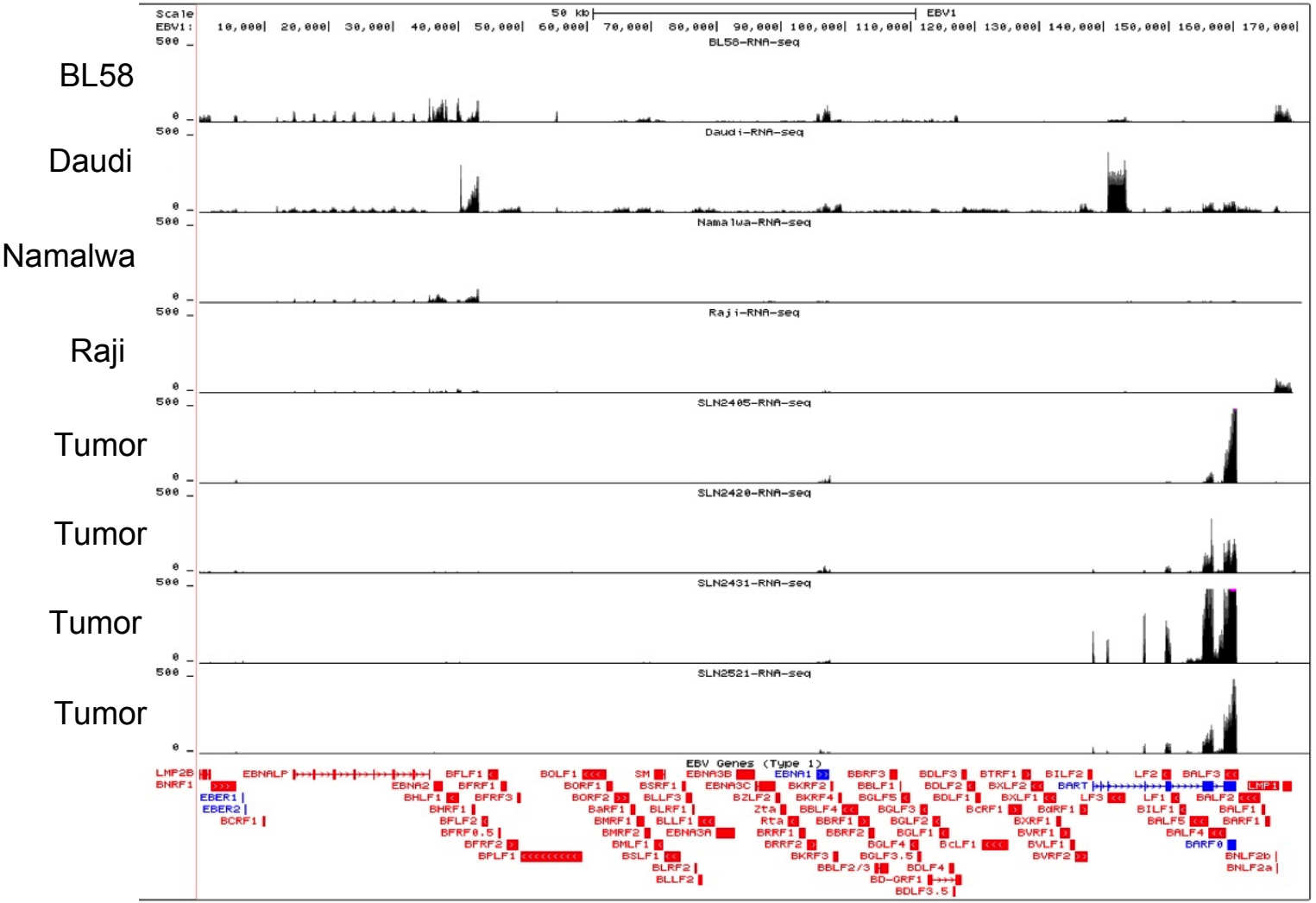


T-EBV + T-EBV - C-EBV +

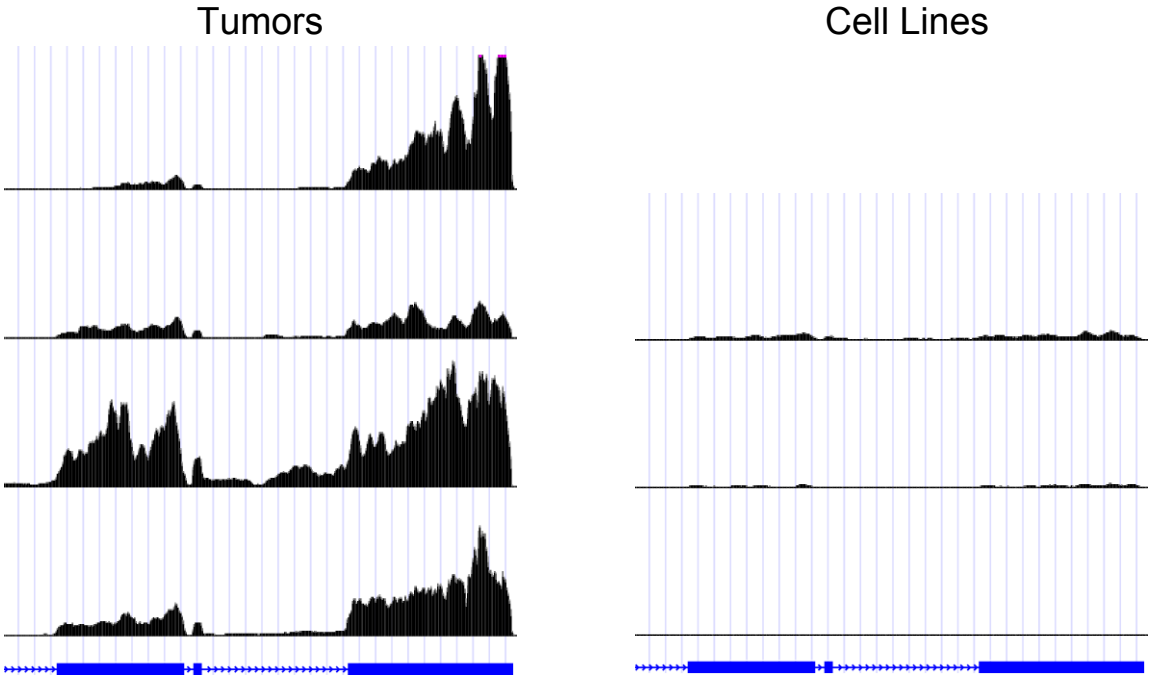
Metagenomic analyses agrees with our read count approach

EBV read counts:
T: 1457
C: 1479

Relative readcounts displayed as a percentage of all reads matched to viruses
Graph from onecodex.com


BL58
Daudi
Namalwa
Raji
Tumor
Tumor
Tumor
Tumor
Scale
EBV1:
50 kb
EBV1
BL58-RNA-seq
Daudi-RNA-seq
Namalwa-RNA-seq
Raji-RNA-seq
SLN2405-RNA-seq
SLN2420-RNA-seq
SLN2431-RNA-seq
SLN2521-RNA-seq
EBV Genes (Type 1)

BART Expression



BART exons 5,6,7

Yamamoto 2012 showed that BART stable transcripts correlate to BART miRNA levels

The cell lines are expressing much lower levels of BART miRNAs

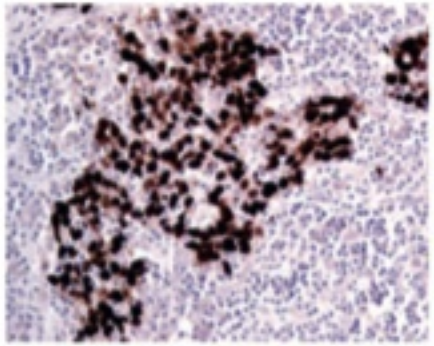
*presumably the signal is higher at this end due to the proximity to the poly-A site

Fully formed question

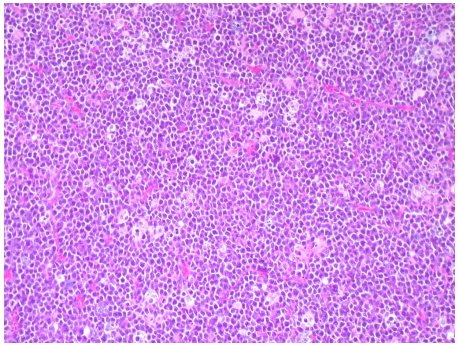
What is leading to the increased expression of the BARTs in BL tumors that is not present in BL cell lines?

Could it be the immune system?

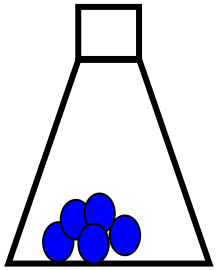
NPC tumor



BL tumor



BL cell line



Inflammatory Infiltrate:	Dense	Modest	None
BART expression	Very High	High	Low/absent

NPC photo source: Tsao et al., “The role of Epstein-Barr virus in epithelial cell malignancies” J Pathol 2015, 235: 232-33.
Burkitt photo source: Ed Uthman, MD. - http://flickr.com/photos/euthman/144136195/in/set-72057594114099781/

How could we determine if it was?

- Step 1: Find evidence of a host response to immune infiltration in RNA sequence data
- Step 2: Attempt to replicate immune environment in cell lines, determine if BARTs go up

Step 1: Differential expression

- Performed differential host gene expression between EBV positive tumors and EBV positive cell lines (Tuxedo pipeline)
- Generated gene list of differentially expressed genes
  - 1326 differentially expressed gene
  - 939 genes upregulated in EBV positive tumors
  - 387 genes downregulated in EBV positive tumors
- Gene lists compared against known biological pathways (DAVID / KEGG)

Enriched pathways

Pathway	Pathway Hits	Pathway Total	Pvalue
Cytokine-cytokine receptor interaction	52	262	2.42E-09
Graft-versus-host disease	12	39	2.10E-04
Complement and coagulation cascades	16	69	3.80E-04
Allograft rejection	10	36	0.0020231065
Antigen processing and presentation	16	83	0.0027974133
Chemokine signaling pathway	26	187	0.0100601475
Natural killer cell mediated cytotoxicity	18	133	0.0445825596
NOD-like receptor signaling pathway	10	62	0.0656116105
TGF-beta signaling pathway	12	87	0.0997428612

- Pathways are defined in KEGG as gene lists
- Differentially expressed genes are compared against pathway lists
- A modified Fisher's exact test is used to determine if the amount of DE genes that match a pathway is significant
- (Only 420 of the DE genes were known to KEGG, and the total number of KEGG genes is 5085)

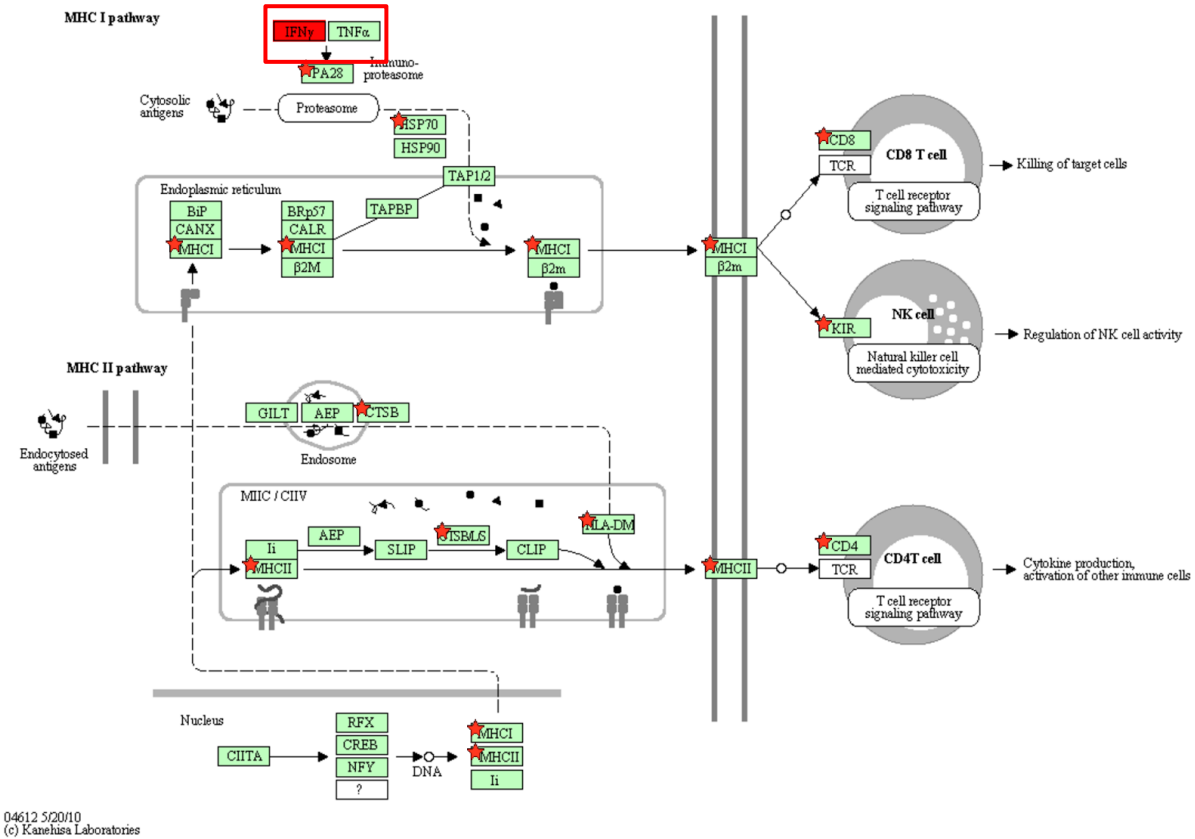
Worked Example - Antigen Processing

	DE List	KEGG “genome”	Total
Antigen Processing	16	83	99
not Antigen Processing	404	5002	5406
Total	420	5085	5505

For this contingency table:
the Fisher exact test value is 0.003426

*DAVID uses a more conservative form of Fisher

Enriched pathways example



Stars signify genes which were present in the differentially expressed gene list

HLA-A (MHCI) was upregulated in EBV positive tumors

How can we accomplish step 2?

- We established that there is evidence of an immune response in the EBV positive tumors
- We now need to pick an experimental condition to expose cell lines too
- We have evidence that interferon gamma is affecting host expression

Interferon gamma is a good starting point

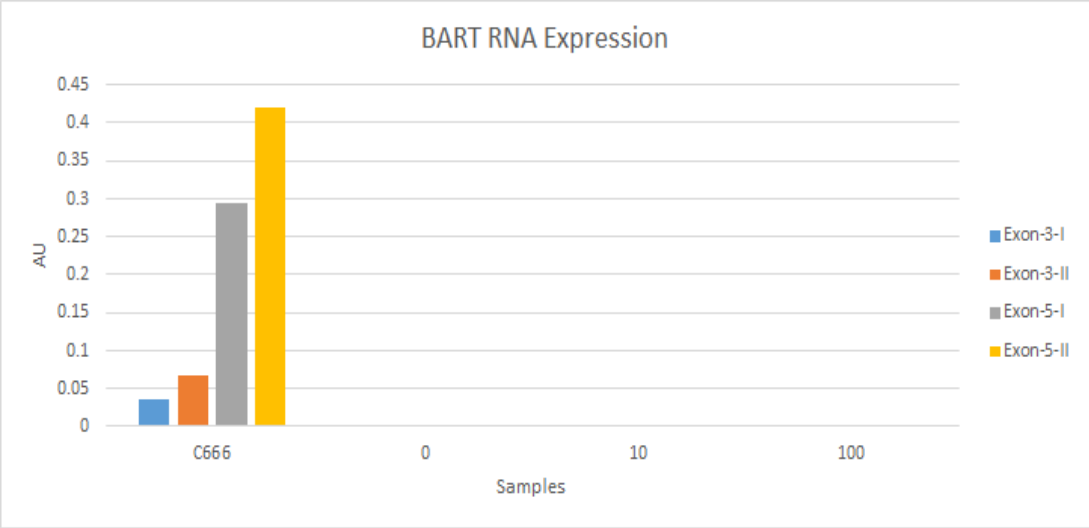
- “We observed marked involvement of the IFNG pathway with 156 of the 490 differentially expressed genes associated with the IFNG pathway”

Strong, M. J., Xu, G., Coco, J., Baribault, C., Vinay, D. S., Lacey, M. R., … Flemington, E. K. (2013). Differences in Gastric Carcinoma Microenvironment Stratify According to EBV Infection Intensity: Implications for Possible Immune Adjuvant Therapy. *PLoS Pathogens*, *9*(5), e1003341. doi:10.1371/journal.ppat.1003341

Can we replicate immune response *in vitro*

- Perform qPCR on EBV positive BL cells, and C666
  - C666 known to express BARTs at high level
  - Akata BL cell lines minimal expression of BARTs
- Treat BL cells with Interferon Gamma (10, 50, 100 ng/ul for 24 hours)
- See if BART expression goes up in response

Results



- This was a very preliminary experiment
- GAPDH values were quite different between conditions
- Still, BART expression appears to be unaffected by Interferon Gamma

Future Experiments

- Identify and assay for a gene known to be upregulated by interferon gamma
  - establish that treatment is effect
- Re-do experiment, control for sample variability
- Test out other cytokines
- Coculture the BL cell lines with Macrophage and/or T Cell lines

Acknowledgments

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- Makoto Ohashi
- Anqi Wang

Questions?