

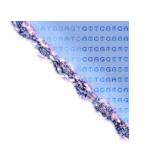
Transcriptome Sequencing Reveals Alternative Splicing Events in Chemotherapy Resistant Colon Cancer Cells

Malachi Griffith

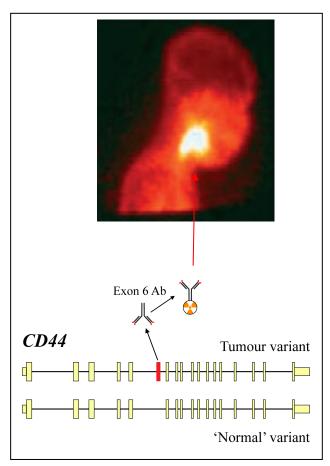
(Marco Marra lab)

AGBT February 5 2009

Biomarker discovery is an important element of cancer research



- Biomarker discovery
 - Differential gene expression
 - Mutation detection
- Diagnosis, prognosis, treatment selection, therapeutic targets
 - e.g. *PSA*, *CEA*
- Specific transcript variants may represent useful biomarkers

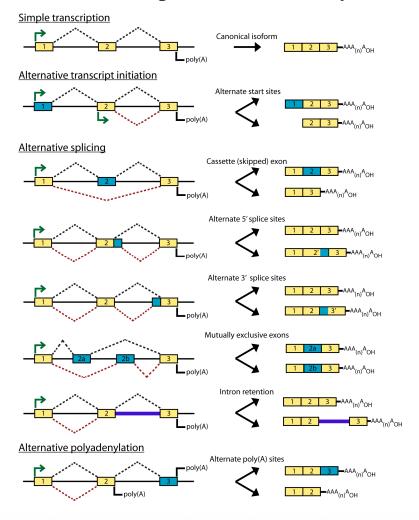


(Venables, 2006)

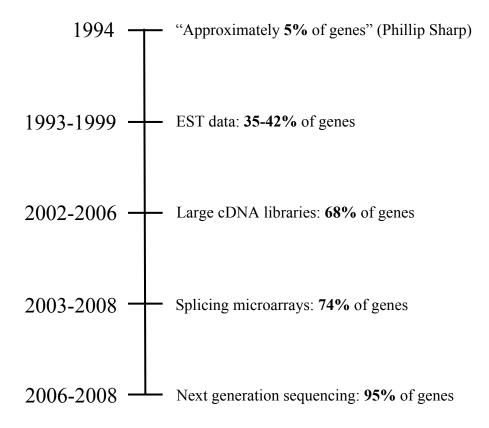
Transcript variation is important to biomarker discovery

TOGAGTOTTOGAGT TOGAGTOGAGTTT CATCAGGGTTT CATCAGGGTTT CATCAGGGTTT CATCAGGGTTT CATCAGGGTTT CATCAGGGTTT CATCAGGGTTT CATCAGGGTTT CATCAGGGTTT CATCAGGTTT CATCAGGTT CATCAGGTTT CATCAGGTT CATCAG

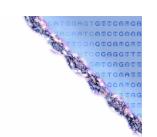
Generates proteome diversity

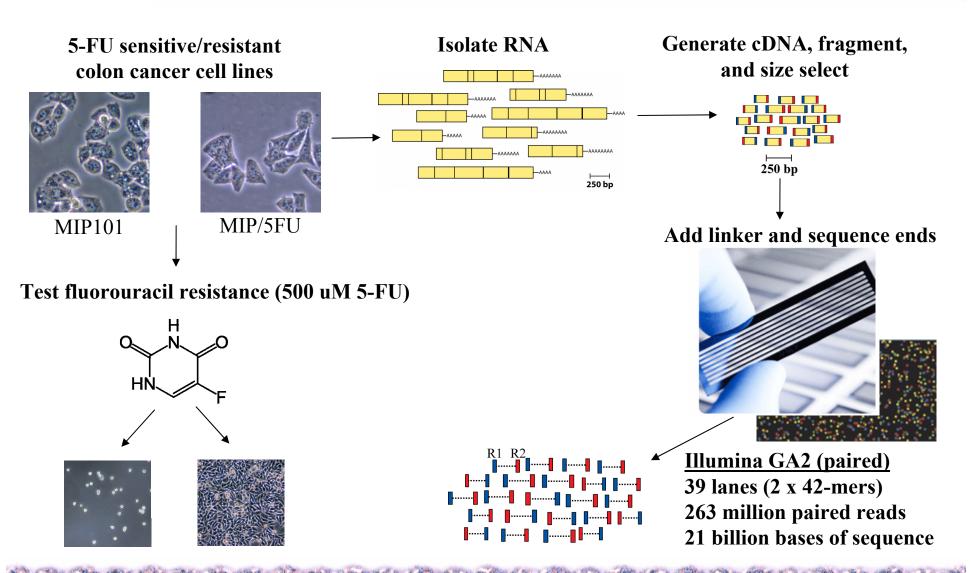


Affects most genes

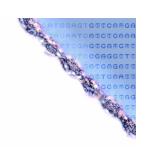


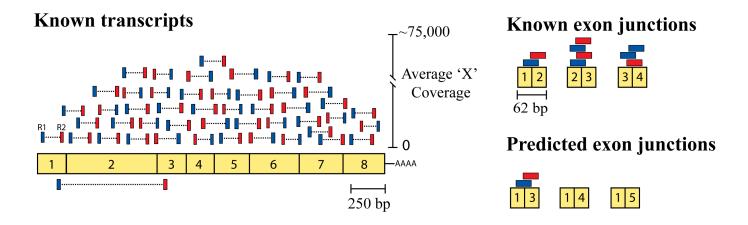
5-FU sensitive and resistant cancer cells were profiled by RNA sequencing





Paired-end reads produced a nearly 'complete' transcriptome sequence

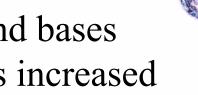


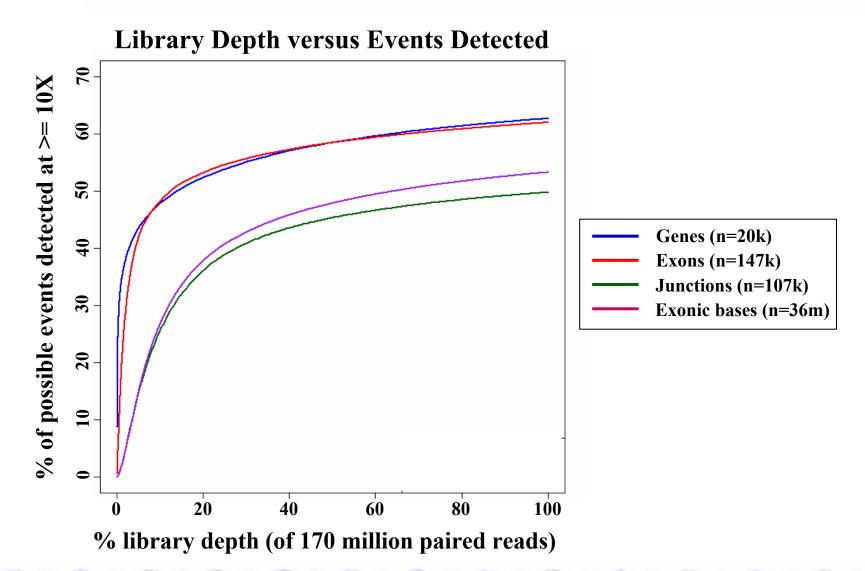


- 70% of all reads map to a known transcript or junction
- Detection by at least 10 reads:
 - 20k genes
 - 147k exons
 - 107k junctions
 - 36m exonic bases positions



Detection of genes, exons, junctions and bases approached a plateau as library depth was increased

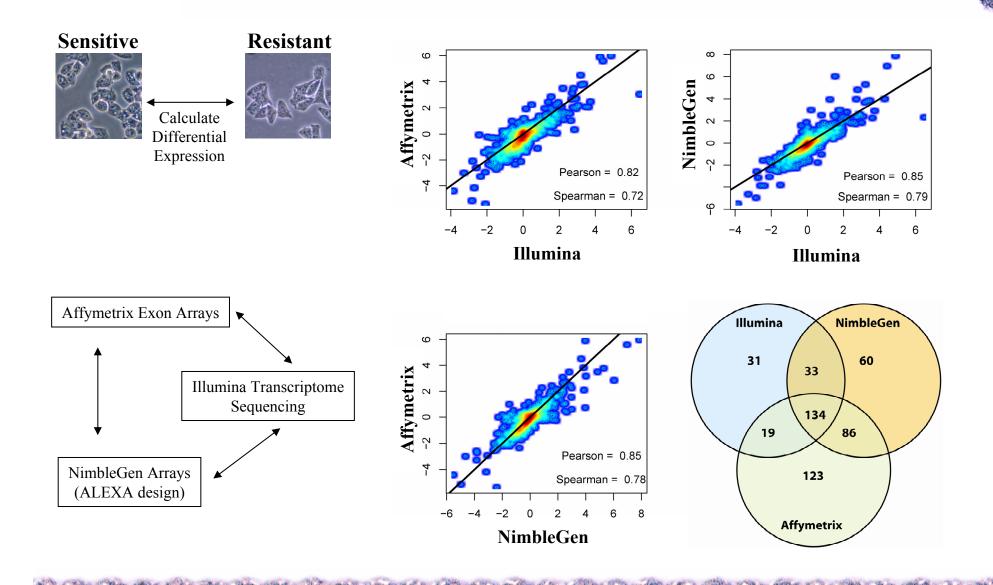






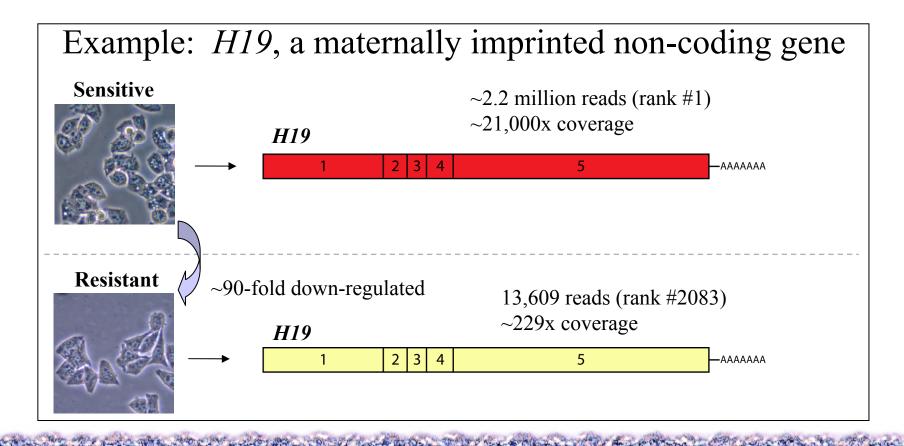
Accurate differential expression estimates were derived

(comparison to Affymetrix and NimbleGen arrays)



Differential expression (DE) of genes and exons was readily detected

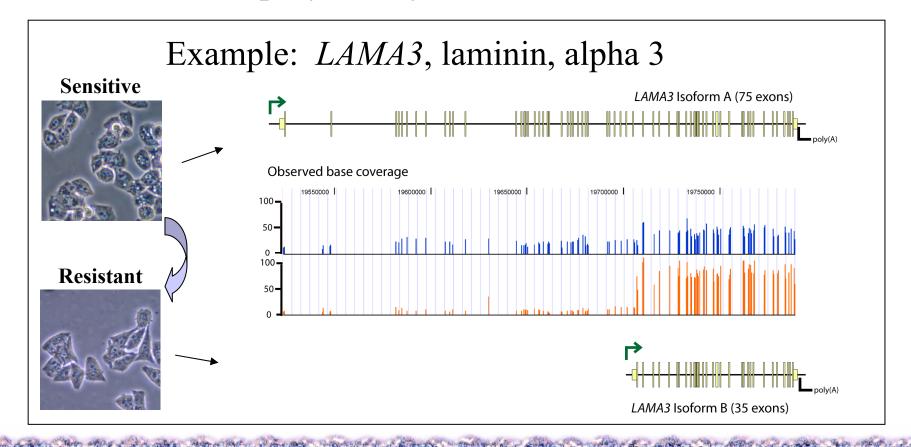
- 32 genes and 299 exons exhibit > 4-fold DE
- Bias towards loss of expression in resistant cells



Differential expression of alternatively transcribed isoforms was also apparent

ATGGAGTOSTORMOS TOGAGCOSTORMOST TOGAGCOSTO TOGAGCOSTO TOGAGCOSTO TOGAGCOSTORMO

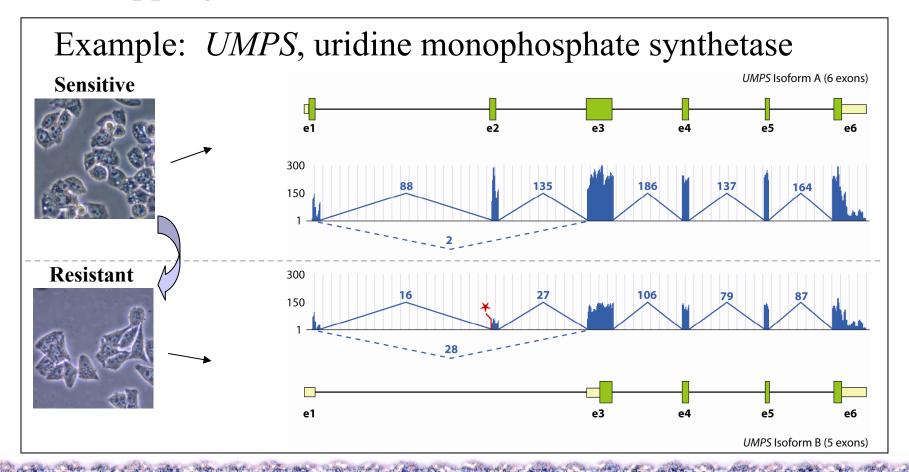
• ~20 genes show evidence of <u>alternative</u> transcript initiation or poly-adenylation



Differential expression of exon-skipping isoforms was also apparent

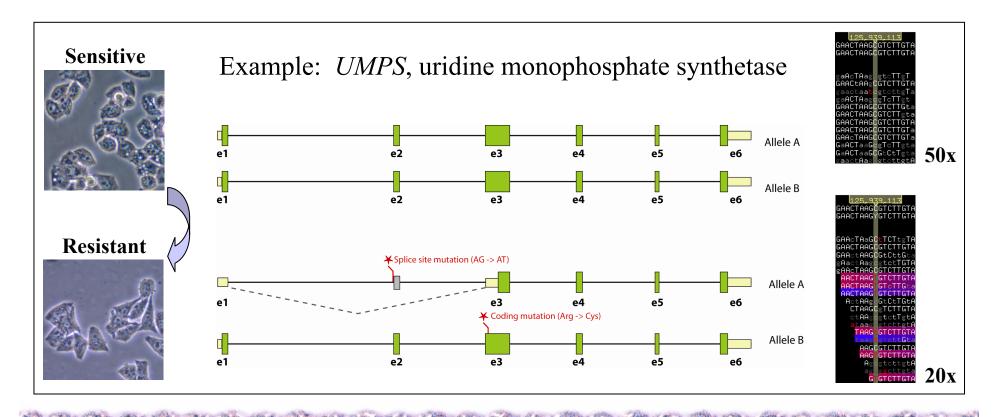
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• 49 genes show evidence of differential exon skipping... 35 known and 14 novel.

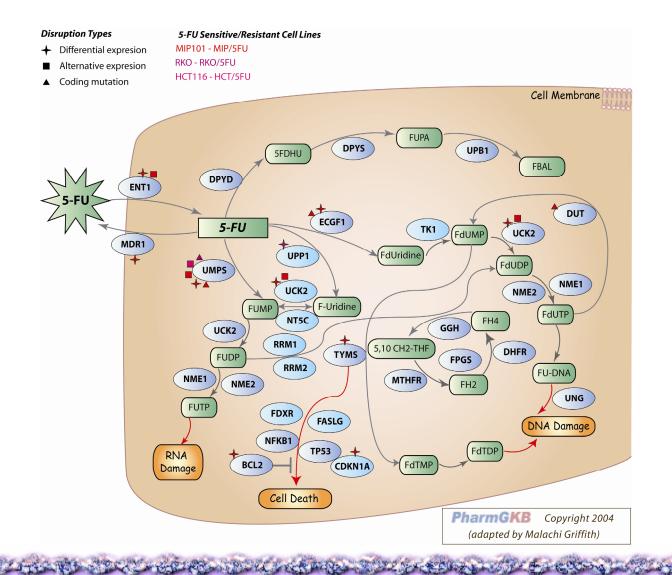


Coding mutations acquired in resistant cells were also identified

• 20-50 genes show evidence of coding mutations acquired in resistant cells



Recurrent disruption of genes known to be involved in 5-FU action was observed



Conclusions

- Massively parallel sequencing allows comprehensive transcriptome re-sequencing
- Allows detection of differences between conditions beyond RNA expression levels
- 5-FU resistance may occur by redundant disruption of drug transport, metabolism and apoptosis pathways
- Clinical validation of *UMPS*
 - cohort of 100 pre- and post-treatment samples

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Affymetrix

Agnes Baross

Susanna Chan

Jennifer Asano

Adrian Ally

Students

Jessica Paul

Lisa Miao

GI cancer

Isabella Tai

Michelle Tang

Clinical

Sharlene Gill

David Owen

Margaret Luk

Carl Brown

Riva Pickering

Technology Development

Patrick Rebstein













ARAGGGCGTTCCAGCGGACACACTATCC TGAGGATCGATTTGACGGAGGCGAGGGTGCAGGT AGTGGCGCAAGCAGGAGAAGAIDGGTGGGCTTGGCGGAGA GATOTOGACG**TGBCGBA886G888GGGGGGG**GTCCTGGGCCAA :TAAGAAAGG**aqacccgbgTcaacmacamabacabcc**gcTagTaat: TORGOGGO886888888888BCMGC86GAC88G88G8AGCAATCCTOG HTAACGAACEEBAGEEBECCEACGGCACGGGBBBGBBBABBACCTCTAGGC AATATATT**385GAAGUMC8MCATTGAGGACTTGG88CMGUMGGG**GGGGTGGCAGT TOCATGG8G#B588G9AG8##5CC58895C888CGC#BG8GG8G#88G6CCCGCAG TOCGACEEMTERECAGGRECEGEGGGGGGAACEGCATGCRATGEBCCCGATT CGGGAGGGGGGAFCATCACCAGC8GC8GC8GCAGCAGGBGCAGCAGGAGCACAA CCAGCIBARAGEGGGGGRAGAGTTCGBBGACTCABCGAABCAGGAAAAACCGATGG CTCCGGCGGCGACGMGGGGGGGGGTTTTGMCATCHCGGGGGGGGGGGCCAGMGAAGGATCGA CTTCARGECESACTGCRCRCCGCGGGAARGGGBABGGGBABBCGCAGGBAGTCGGCGG CTCCBBGBGGGTCGGBTBAMGCBBBBGBMBBBBGBCCGBABBGGATBGGBABBFCCTC TGGCHBBGBHGGAGGGBBABCCCBABABBBCCCBCBCCTTTTTGGCCBGCGBAAGTC **ССССТОНОМБИНОВИТАССОВИНОВИНОВИНОВИТЕЛЬНО** CCAGBBTBAGBBCGGCCCABTBGBABBBBCBBCBBCBBCBBCBBCBBBTGAAT астт**жадацияттсявяцццаваевысцаансцаансцаа**саасаасаастт ATTCTTBGMCTCT88GCA8GGGT88CBTCT8MGGCC88G8CC8AAC8M8GCCGCCAC TGACT88M6M7AT**T633AM83M886GM88C88888CCCCM886**CCT88M**68C**TAGTTTF TGAAGTMGCACTAGBCCMACCCTGGASMCGCATCTSAAGAAATAGAABMGTGGTTTA AAAGAGMMCGGGTAAGMGTEBAACMTGTTBEBEBCAACGAGAATTGTTBEBAAAAGAAT TTTCGTA**BG8C8G8TTTGTG8ATTTC88M888CC8GAAT**CG**88MCG88M**CGAAAC остасс**стиндининавадилаттт**дт**дданиаваитабник**овсаадстас :AATGGATT**HGGGCHTCBAGBBGG56TTHAGH8GGCGH8C**AACATTTGC TACCAAAG**AGAGGCEEBAAGGGGAACGGMGAGGGCG**CCCACTGGT ACTOCTATTAACGEGEBTCMGCEGEGUMECTGATCCTAAG A C G C T C C A T T A A G T G A A A A G C T C A C A G C A G A G A T G A T A TOAGTTAGTTCTAAAGTACTACAATAGATAT

Observed distribution of fragment sizes and relative transcript position

