Genomic profiling of budding yeast resistance to food carcinogens underscore the importance of DNA damage tolerance pathways in avoiding mutations

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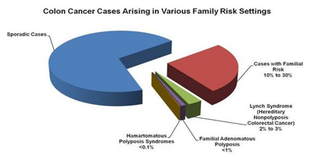
Abstract

The human response to environmental carcinogens is highly variable. Environment, lifestyle, and genetics are factors that influence bioactivation. Genetic factors include polymorphic P450 and DNA repair genes; however, epidemiological studies may lack significance due to inadequate patient numbers. We used budding yeast as a model organism to determine genetic susceptibility to food-associated carcinogens, including benzopyrene (BaP), aflatoxins (AFB1) and heterocyclic aromatic amines (HAAs), such as 2-amino-3-methylimidazo[4,5-f]quinoline (IQ). Budding yeast does not contain P450s that activate these compounds, so we introduced expression vectors that contain specific human P450 and NAT2 genes. In yeast, either CYP1A2 or CYP1A1 activates AFB1, while both CYP1A2 and NAT2 are required for activation of IQ. To measure genotoxic effects, we measured recombination and mutation frequencies, Rad51 foci, growth inhibition and DNA adducts. To determine resistance genes, we used a high throughput approach for screening the yeast deletion library expressing specific P450 genes or expressing no P450 genes. Screens for aflatoxin resistance in the collection expressing CYP1A2 identified 31 genes, including checkpoint and RNA metabolism genes, several of which have human orthologues are mutated in cancers. Screens for aflatoxin resistance from the deletion collection expressing no CYPs identified CTR1, a gene that functions in high-affinity copper transport. Interestingly, this gene has been identified in screens for profiling yeast resistance to the fungicides captafol and folpet. We are now performing screens to identify genes involved in resistance to IQ. Preliminary data identified both recombinational repair and DNA damage tolerance genes. Further high throughput analysis will be performed using other food carcinogens, including 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx). These screens provide a novel methodology for identify genes that confer resistance to P450-activated toxicants. Grant Support: National Institutes of Health, 1R15ES023685-01.

Background

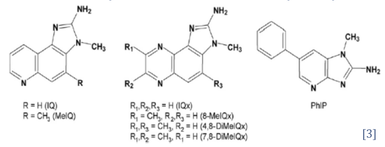
- Colorectal Cancer is the fourth leading cause of cancer death (2014)
  - Estimated ~50,000 deaths [1]
- Liver cancer is the sixth leading cause of cancer death (2012)

Colon Cancer Cases Arising in Various Family Risk Settings



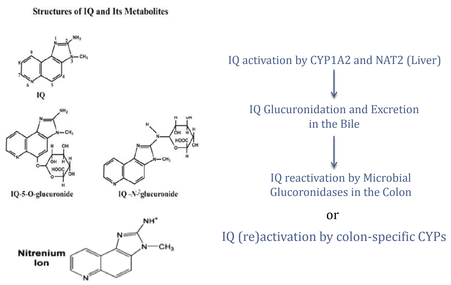
[2]

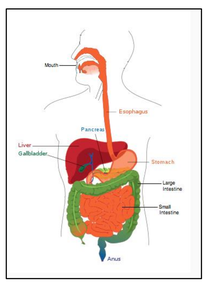
Heterocyclic Aromatic Amines (HAAs)



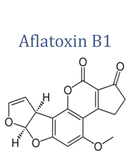
[3]

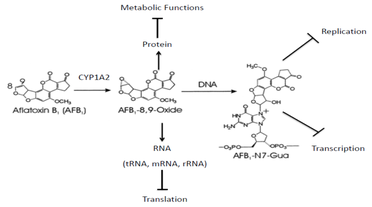
Mechanisms of Activation of IQ by Phase I and II Enzymes





Mechanisms of Activation of AFB1





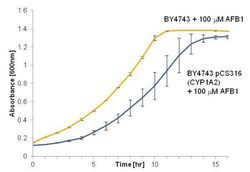
[4]

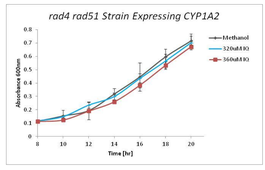
Hypothesis

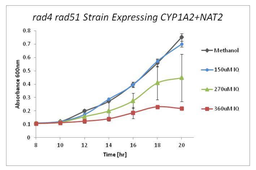
Whereas a small fraction of cancer is caused by high-penetrant genes, the majority of sporadic cancer is caused by a combination of interactions between low penetrant – gene environment interactions.

Methods

Confirm Genotoxicity of P450-Activated Compounds In Yeast

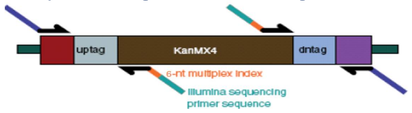






Construct Yeast Libraries Containing The Entire Deletion Collection

- >5,000 strains, each strain contains a deletion for a single gene
- Each gene deletion is marked by an antibiotic cassette and two molecular bar codes (identifiers)
- Quantified by detecting the molecular bar codes, amplifying via PCR and sequencing the barcodes
- Human CYP1A2 has been introduced into 90% of the collection
  - Currently introducing CYP1A2 and NAT2 genes into another pool



Results

- Raw sequencing data is run through a bioinformatics regiment to demultiplex, trim, and count the barcodes
  - Yeilds a count of each mutant pre and post exposure
  - Further bioinformatics processing compares counts of treated vs. control exposures

Output of Bioinformatics Processing

AFB1 Sensitive Genes - Screen 1

Gene ID	m.value	p.value	q.value	Gene Name
YBL107C	-4.06328	7.34E-14	4.35E-10	MIX23
YDR322W	-4.28367	3.08E-13	9.14E-10	MRPL35
YBR270C	-3.96333	7.46E-12	1.47E-08	BIT2
YDR245W	-4.30245	1.07E-11	1.59E-08	MNN10
YDR133C	-4.53444	1.63E-11	1.94E-08	Dubious
YER005W	-4.42756	2.03E-11	2E-08	YND1
YIR016W	-4.55955	3.29E-11	2.78E-08	unknown
YOR268C	-4.15813	2.7E-10	1.96E-07	unknown oxidative stress
YDR076W	-3.97622	2.97E-10	1.96E-07	RAD55
YPL167C	-4.15215	4.04E-10	2.39E-07	REV3
YNL012W	-3.51771	1.02E-09	5.48E-07	SPO1
YOR347C	-3.9434	1.71E-09	8.44E-07	PYK2
YER007C-A	-3.17638	2.17E-09	9.88E-07	TMA20
YDR051C	-4.34985	4.87E-09	2.06E-06	DET1
YCR083W	-4.37389	6.62E-09	2.62E-06	TRX3
YOR346W	-4.06683	1.32E-08	4.89E-06	REV1
YBR059C	-3.05176	1.51E-08	5.26E-06	AKL1
YOR368W	-4.23719	2.77E-08	9.13E-06	RAD17
YNR032W	-2.78494	2.93E-07	8.95E-05	PPG1
YDR221W	-2.67674	3.02E-07	8.95E-05	GTP1
YDR346C	-2.6069	8.95E-07	0.000253	SVF1
YPL271W	-3.45619	1.09E-06	0.000292	ATP15
YCR062W	-3.35996	1.13E-06	0.000292	unknown
YBR044C	-4.00454	1.75E-06	0.000432	TCM62
YDR384C	-4.18208	3.93E-06	0.000931	ATO3
YER162C	-2.36977	7.69E-05	0.015718	RAD4
YJR019C	-1.9487	7.69E-05	0.015718	TES1
YNL068C	-1.70355	0.000103	0.020404	FKH2
YML014W	-2.31801	0.000119	0.022765	TRM9

AFB1 Sensitive Genes - Screen 2

Gene ID	m.value	p.value	q.value	Gene Name
YGR258C	-2.5765	7.28E-17	4.31E-13	RAD2
YML095C	-2.34872	1.03E-10	3.04E-07	RAD10
YPL022W	-2.00868	1.91E-10	3.78E-07	RAD1
YIL030C	-1.66932	3.45E-09	5.11E-06	SSM4
YLR074C	-1.08013	2.86E-05	0.084826	BUD20
YKR046C	-7.89072	3.23E-06	0.001277	PET10
YIL132C	-1.25308	0.00072	0.079022	CSM2
YMR312W	-1.01899	0.001032	0.09526	ELP6
YMR283C	-0.91525	0.000755	0.080721	RIT1
YLR032W	-2.5585	1.32E-06	0.000653	RAD5
YMR264W	-1.09343	0.000236	0.037824	CUE1

NOTE: CSM2 is highlighted to show downstream results processing of gene ontology groups

Key:

- m.value – fold change from solvent exposure (DMSO) to carcinogen (AFB1)
- p.value - statistical p-value
- q.value – p-value adjusted for the three biological replicates

- Output list of significant genes is copied into FunSPEC
  - Organizes data into gene ontology groups, allows for identification of additional genes in pathways that may be sensitive

FunSpec

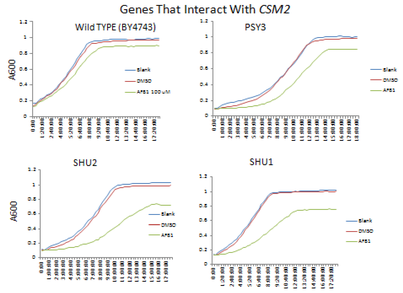
GO Biological Process (2062 categories)

Category	p-value	In Category from Cluster
response to DNA damage stimulus	3.69E-06	RAD55 RAD51 RAD4 RAD2 CSM2 RAD5 RAD52 RAD10 IES4 REV1 RAD17 RAD1 REV3
DNA repair	9.40E-06	RAD55 RAD51 RAD4 RAD2 CSM2 RAD5 RAD52 RAD10 REV1 RAD17 RAD1 REV3
double-strand break repair via single-strand annealing	9.38E-05	RAD55 RAD51 RAD52
nucleotide-excision repair, DNA incision, 5'-to lesion	0.0002005	RAD10 RAD1
meiotic joint molecule formation	0.000696	RAD51 RAD52
DNA recombinase assembly	0.000696	RAD55 RAD52
DNA metabolic process	0.0007206	RAD55 RAD51 RAD1
double-strand break repair via single-strand annealing, removal of nonhomologous ends	0.001181	RAD10 RAD1
removal of nonhomologous ends	0.001181	RAD10 RAD1
meiotic DNA recombinase assembly	0.00195	RAD55 RAD52
error-free translesion synthesis	0.002898	REV1 REV3
error-prone translesion synthesis	0.002898	REV1 REV3
meiotic mismatch repair	0.00531	RAD10 RAD1
heteroduplex formation	0.00631	RAD55 RAD51
sphingolipid metabolic process	0.01014	YND1 NCR1
postreplication repair	0.01412	RAD5 RAD52
mitotic recombination	0.01412	RAD10 RAD1
cellular response to oxidative stress	0.01491	TRX3 SVF1 MCR1 TSA1
chronological cell aging	0.02114	DNM1 TRM9
DNA damage checkpoint	0.02373	TSA1 RAD17
tRNA methylation	0.0293	TRM9 TRM44
telomere maintenance via recombination	0.0293	RAD51 RAD52
electron transport chain	0.03213	TRX3 QCR6 FRE5
lipid transport	0.03633	DET1 NCR1
double-strand break repair	0.04179	RAD5 RAD17
tRNA wobble uridine modification	0.04618	TRM9 ELP6

GO Molecular Function (1646 categories)

Category	p-value	In Category from Cluster
damaged DNA binding	6.87E-06	RAD51 RAD4 RAD10 REV1 RAD17
single-stranded DNA specific endodeoxyribonuclease activity	9.38E-05	RAD2 RAD10 RAD1
tRNA (uracil) methyltransferase activity	0.0002005	TRM9 TRM44
recombinase activity	0.000696	RAD51 RAD52
single-stranded DNA binding	0.001021	RAD51 RAD2 RAD10 RAD1
nuclease activity	0.001925	RAD2 RAD10 NGL3 RAD17 RAD1
DNA strand annealing activity	0.00195	RAD52 RAD10
endonuclease activity	0.00398	RAD2 RAD10 NGL3 RAD1
DNA-dependent ATPase activity	0.00781	RAD55 RAD51 RAD5
DNA-directed DNA polymerase activity	0.02114	REV1 REV3
exonuclease activity	0.04179	NGL3 RAD17

According to SGD, CSM2 (YIL132C) is a Component of SHU complex. This complex also includes PSY3, SHU1, SHU2, and promotes error-free DNA repair [5]



Final result is compiled list of gene knockouts that confer sensitivity to AFB1

- Current work underway with HAAs

RECOMBINATIONAL REPAIR	NUCLEOTIDE EXCISION REPAIR	DNA DAMAGE TOLERANCE/ REPLICATION BYPASS	MISMATCH REPAIR	CHECKPOINT RESPONSE	MODULATION OF THE CHECKPOINT RESPONSE	DNA REPLICATION/ FORK INTEGRITY
RAD51	RAD2	RAD5	MLH1	MEC1	PPH2	MUS81
RAD52	RAD14	RAD18	MSH3	TEL1	PSY2	SGS1
RAD55	RAD10	REV1	MSH4	RAD9		RTT109
RAD54	RAD1	REV3	MSH6	RAD53		RAD27/FEN1
MRE11		RAD30		DUN1		ESC2
		UBC13		RAD17		MPH1
		RAD6				POL32
		MMS2				

** AFB1 Sensitivity

Conclusions

- The CYP1A2+NAT2 gene combination results in higher HAA genotoxicity
- Yeast DNA repair mutants expressing CYP1A2 and CYP1A2+NAT2 show sensitivity to HAAs (IQ) and AFB1. HAA genotoxicity increases when NAT2 is expressed
- High-throughput screens indicate DNA repair, checkpoint control and recovery, mitochondrial maintenance, RNA modification are all involved in conferring AFB1 resistance
- Recombinational repair and mutation avoidance are key processes in AFB1 resistance

Acknowledgements

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References

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