Computational Approach for Discovery and Characterization of Cancer Driver Missense Mutations

ACS Spring 2020 Virtual National Meeting
3/23/2020

G. Andrés Cisneros
@CisnerosRes

Acknowledgements

Group Members

Current

Dr. Robert E. Duke
Dr. Sehr Naseem-Khan
Erik A. Vázquez
Mark A. Hix
Emmett Leddin
J. Enrique Vázquez
Madison Berger
Ally Smith
Miguel Espinoza
Cory Shen
Krithika Ravishankar

Alumni (contributing)

Dr. Alice Walker (Stanford) Dr. Pavel Silvestrov Dr. Dong Fang (Xtalpi)
Dr. Hedieh Torabifard (U of M) Dr. Rebecca Swett (Vertex)
Dr. Hatice Gokcan (UNC) Dr. Eric Kratz (Continental)

Collaborators

Linda Chelico (U. Saskatchewan)
Penny Beuning (Northeastern U.)
Robert Hausinger (MSU)
Rahul Kohli (U. Penn)
Jean-Philip Piquemal (Sorbonne U.)

Gregory Dyson (Karmanos)
Iannis Aifantis (NYU Med)
Faruck Morcos (UT-Dallas)
Tom Darden (OpenEye)
Matt Allen (WSU)

Stas Groysman (WSU)
Berny Schlegel (WSU)
Judy Wu (U. Houston)
Pengyu Ren (UT-Austin)
Oleg Tsodikov (UKY)

Funding

Computing Time

Outline

- Discovery and Characterization of Cancer—associated missense variants
- Characterization of DNA Polymerase cancer mutants
 - DNA Polymerase λ breast cancer-associated variant
- DNA demethylases
 - ALKBH7 prostate cancer-associated variant
- APOBEC3H; lung cancer associated variant
 - Characterization of cancer variant and prediction of rescue mutant

From Bioinformatics to Electronic Structure

- New computational approach to uncover and characterize disease-associated mutations:
- Targeted search for missense mutations combined with statistical analysis
- Investigation of structural/functional/dynamical effects of cancer variants by Molecular Dynamics (MD) simulations and/or hybrid (QM/MM) simulations

DNA Polymerases

<https://dnalc.cshl.edu/resources/3d/04-mechanism-of-replication-advanced.html>

- QM/MM investigation of reaction mechanism of DNA Pol λ
- Predicted catalitically active “2nd Shell” residues around active site (GAC, *et al*, *DNA Rep.*, **2008**, 7, 1824)

- Involved in DNA replication, repair and recombination
- Replicative polymerases synthesize DNA at 50 nb/s rate (10 h for full human genome replication)
- 1 in 10 billion BP error rate

GAC, *et al*, *DNA Rep.*, **2008**, 7, 1824

Single Nucleotide Polymorphism (SNP) Analysis

- **Hypothesis:** *Individuals with SNPs on DNA polymerase genes that affect “2nd shell” catalytically active residues with a functional impact on the protein product are likely to have a predisposition toward cancer*
- **Approach:** Develop an algorithm (and software) to search for SNPs on a single gene(s) of interest, ascertain statistical significance of SNPs → Hypothesis Driven SNP Search (HyDn-SNP-S); followed by computational characterization of structure/function/dynamics impact

Linking Phenotypes to Structures

- Application of HyDn-SNP-S to search and characterize missense cancer SNPs on human DNA Pols

Cancer SNPs on DNA Pols

SNPs in DNA Pols

Polymerase	Mutations	Phenotypes
Pol α 2	F548L, G592V	melanoma, breast, pancreas, prostate,
Pol β	H51Q, K54Stop, R137Stop, G144G, P242A, Y296F	melanoma, prostate,
Pol Δ 2	T436A, S412C, R166A, Y54Stop	melanoma, prostate,
Pol Δ 3	A215G, A124V	melanoma, prostate,
Pol Δ 4	Q37R, R39S, R39G	melanoma, prostate,
Pol ϵ 3	L7V	prostate
Pol η	T478S, T478M, S514A, Q524Q, D646V	melanoma, prostate, lung
Pol λ	R437W, R438W	melanoma, breast, pancreas, prostate

R438W Affects Loop 1 Dynamics

- R438W is located near loop 1
- Loop 1 is critical for Polλ fidelity
Bebenek *et al.*, NAR, **38**, 5419, 2010
- MD on 4 systems to study cancer variant effect

Complex	WT	Mutant
Polλ-DNA	1RZT	1RZT R438W
Polλ-DNA-dNTP	2PFQ	2PFQ R438W

- Motion of loop 1 is correlated to site 438
- R438W alters loop 1 motion
- Loop 1 motion change in R438W may affect Polλ fidelity

R438W Confirmed as Breast Cancer Risk Factor

Molecular Cancer Research

[Advanced Search](#)[Home](#)[About](#)[Articles](#)[For Authors](#)[Alerts](#)[News](#)

DNA Damage and Repair

Estrogen Drives Cellular Transformation and Mutagenesis in Cells Expressing the Breast Cancer–Associated R438W DNA Polymerase Lambda Protein

Antonia A. Nemec, Korie B. Bush, Jamie B. Towle-Weicksel, B. Frazier Taylor, Vincent Schulz, Joanne B. Weidhaas, David P. Tuck, and Joann B. Sweasy

DOI: 10.1158/1541-7786.MCR-16-0209 Published November 2016

AlkB Family Enzymes

- In charge of dealkylating damaged nucleobases
- Fe(II)/ α -ketoglutarate dependent
- Can act on different substrates: ssDNA, dsDNA, RNA, even some proteins
- Conserved active site motif
- AlkB (*E coli*) title enzyme, and 9 human homologues

Cancer SNPs on AlkB family

Are there any SNPs on AlkB enzymes that are correlated in a statistically significant manner to prostate cancer?

SNP	Gene	Intronic(I)/ Exonic(E)	Effect of the Missense Mutation
rs7160307	ALKBH1	I	R191/Q
rs3751812	FTO	I	
rs6499653	FTO	I	
rs8044769	FTO	I	
rs7540	ALKBH7	E	
rs7193938	FTO	I	
rs9302652	FTO	I	
rs7190492	FTO	I	
rs8050136	FTO	I	
rs12447481	FTO	I

Location of R191 w.r.t. Active Site

- R191 is ~ 22 Angstrom away from active site
- MD simulations on wild type (WT) and R191Q mutant with α KG and succinate bound to Fe(II)
- 500 ns (in triplicate) for each system: AMBER14/ff12SB.
- MMGBSA to calculate change in co-factor/co-substrate binding enthalpy
- UV/Vis absorbance on WT and R191Q

Comparison of WT and R191Q Structure

Gray: WT ALKBH7
Yellow: R191Q ALKBH7

R191Q ALKBH7
eliminates hydrogen
bond network on
loop, resulting in
structural change in
active site

Co-Factor/Co-Substrate Binding Energy (MMPBSA)

Difference Absorption Spectra of ALKBH7

Wild Type

R191Q Variant

Data from Dr. T. Müller and Prof. R. Hausinger,
Thanks to Prof. Z. Chen for ALKBH7 Plasmid

HyDn-SNP-S on DNA Repair/Modification Enzymes

	breast	prostate	lung	pancreatic	CLL	DLBCL	FL	MZL
ABH7		rs7540						
MSH3			rs184967 rs26279					
POLE					rs4883543 rs4883544			
POLA2								rs487989
POLD1							rs1726801	
POLG						rs2307441		rs3087374
POLG2		rs1427463						
POLI		rs8305						
POLL	rs3730463				rs3730476	rs3730463 rs3730476	rs3730476	
POLN	rs9328764 rs10011549 rs1001878							rs11937432 rs2353552
POLQ	rs3218651	rs3218651		rs3218651	rs3218642			
REV3L			rs462779					
TET1	rs12221107		rs12221107 rs16925541		rs3998860			rs3998860
APO2						rs2076472	rs2076472	
APO3G			rs8177832					
APO3H			rs139298		rs139297	rs139297		
APO4				rs1174657 rs1174658		rs16861394		
MBD4				rs140693		rs2307293		
MUTYH						rs3219484	rs3219484	rs3219484
NEIL2					rs8191613 rs8191664			
NEIL3		rs13112390 rs13112358	rs13112358	rs1876268	rs1876268	rs13112358 rs13112390 rs1876268		
APEX1							rs1130409	
APEX2						rs2301416		
PARP1			rs1136410				rs1136410	
PARP2				rs3093921				rs3093921

APOBEC3H

- Member of the APOBEC cytidine deaminases
- A3H catalyzes C to U deamination in ssDNA and mRNA
- Responsible for antiretroviral activity against HIV-1
- HyDn-SNP-S uncovered A3H K121E variant related to lung cancer
- MD on en WT A3H and K121E, K117E and K121E/K117E A3H
- 200 ns (in triplicate) for each system: OpenMM/ff12SB

K121E Forms New H-Bond Networks

K117E/K121E rescues; K117E stabilizes dimer

Diff. RMSF w.r.t. WT

PCA (gray=WT)

Diff. Correlation

Experimental Results

Expression

Transient expression in 293T Cells

Activity

Dimerization

Data from Prof. L. Chelico, B. Flath, and L. Wong

Experimental Results

γ H2AX foci

Data from Prof. L. Chelico, B. Flath, and L. Wong

Summary

- HyDn-SNP-S: New SNP mutant discovery and characterization approach using atomistic simulations
- Uncovered and characterized various missense mutations on DNA Pol λ associated with breast cancer
- ALKBH7 prostate cancer mutations results in ALKBH7 R191Q variant, affects co-substrate binding
- A3H K121E is associated with lung cancer, destabilizes the structure and abolishes catalytic activity
- Active A3H coupled with A3B exhibit increased DS breaks, elimination of A3H gives “just right” mutation rate

Acknowledgements

Group Members

Current

Dr. Robert E. Duke
Dr. Sehr Naseem-Khan
Erik A. Vázquez
Mark A. Hix
Emmett Leddin
J. Enrique Vázquez
Madison Berger
Ally Smith
Miguel Espinoza
Cory Shen
Krithika Ravishankar

Alumni (contributing)

Dr. Alice Walker (Stanford) Dr. Pavel Silvestrov Dr. Dong Fang (Xtalpi)
Dr. Hedieh Torabifard (U of M) Dr. Rebecca Swett (Vertex)
Dr. Hatice Gokcan (UNC) Dr. Eric Kratz (Continental)

Collaborators

Linda Chelico (U. Saskatchewan)
Penny Beuning (Northeastern U.)
Robert Hausinger (MSU)
Rahul Kohli (U. Penn)
Jean-Philip Piquemal (Sorbonne U.)

Gregory Dyson (Karmanos)
Iannis Aifantis (NYU Med)
Faruck Morcos (UT-Dallas)
Tom Darden (OpenEye)
Matt Allen (WSU)

Stas Groysman (WSU)
Berny Schlegel (WSU)
Judy Wu (U. Houston)
Pengyu Ren (UT-Austin)
Oleg Tsodikov (UKY)

Funding

Computing Time