Somatic mutations, genome mosaicism, aging and disease

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Disclosure:

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Stochastic and adaptive changes in aging

Environment

Endogenous

Stochastic changes

Adaptive changes

Aging

Different change in each cell

Same change in all cells
AN ATTEMPT AT A RATIONAL CLASSIFICATION OF THEORIES OF AGEING

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THE MAINTENANCE OF THE ACCURACY OF PROTEIN SYNTHESIS AND ITS RELEVANCE TO AGEING

L. E. Orgel

ON THE NATURE OF THE AGING PROCESS

Leo Szilard

Protein mistranslation

Aging

Somatic mutations

Impaired gene expression

Redundancy

Aging
Progress in single-cell proteomics

Slavov, N., Curr Opin Chem Biol 2021
Random mutations affect predominantly gene regulatory sequences

Milholland, Suh, Vijg, Exp. Gerontol. 2017
Somatic DNA mutations: causes and consequences

DNA damage

Errors during repair or replication

DNA mutations

Original allele

Mutated allele

Germline
Diversity and perpetuation of life

Soma
Aging?
How to measure somatic mutations?
Missing Y metaphases in bone marrow of males

Sakurai and Sandberg, Cancer, 1976
Chromosomal abnormalities and aging

Ramsey et al., Mut Res 1995
Telomere length declines with age

Age-related tissue-specific mutation accumulation in lacZ reporter mice

Brain

\[ y = 0.0467x + 3.9769 \]
\[ R^2 = 0.1373 \]

Heart

\[ y = 0.2728x + 4.2501 \]
\[ R^2 = 0.5384 \]

Liver

\[ y = 0.2915x + 4.0809 \]
\[ R^2 = 0.5361 \]

Small intestine

\[ y = 0.6154x + 8.655 \]
\[ R^2 = 0.6442 \]

Dollé et al., Nat Genet 1997

Dollé et al., PNAS 2000
Aging-associated accumulation of point mutations and genome rearrangements in organs of mice

Estimated number of mutational events per cell

<table>
<thead>
<tr>
<th></th>
<th>Point mutations</th>
<th>Genome rearrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young</td>
<td>Old</td>
</tr>
<tr>
<td>Brain</td>
<td>50</td>
<td>74</td>
</tr>
<tr>
<td>Liver</td>
<td>39</td>
<td>113</td>
</tr>
<tr>
<td>Heart</td>
<td>37</td>
<td>103</td>
</tr>
<tr>
<td>Small intestine</td>
<td>89</td>
<td>332</td>
</tr>
</tbody>
</table>

Dollé et al., Genome Res. 2002
Exponential increase of the number of exome mutations in tumors with age of the patients

Whole-exome sequencing
32 tumor types, 6,969 patients
p<2.2*10^{-16} (linear model)
Data from TCGA

Milholland et al., Oncotarget 2015
Single cell clonal expansions can be sequenced but only for those cells that are mitotically active.

Expand stem cells *in vitro*

Deep sequencing of natural single cell expansions from micro-biopsies

WGS: whole genome sequencing
Gold standard: whole genome sequencing of single cells after amplification

Optimization of variant calling

Single-cell multiple displacement amplification (SCMDA): validation

Dong X, Zhang L et al. Nat. Meth., 2017
A genome-wide index for somatic mutations

Single Nucleotide Variant  Deletion  Insertion  Tandem Duplication  Inversion  Translocation

SNVs 1 bp  Small insertions and deletions (1-50 bp)  Structural variants ≥50 bp

SCcaller v2.0

SCcaller_PEA
Dong X, et al., in progress
Single nucleotide variants (SNVs) accumulate at different rates in different tissues during aging.

Fitted model: SNV = a + Age^b

Zhang et al., PNAS 2019; Brazhnik, Sun et al., Sc. Adv. 2020
Huang, unpublished; Sun, unpublished
Is there a functional impact of somatic mutations?
Negative selection indicates a damaging effect of somatic mutations in aging

Zhang et al., PNAS, 2019
Pathogenic mechanisms of somatic mutations in aging

Vijg, Dong, CELL 2020
LETTERS

Increased cell-to-cell variation in gene expression in ageing mouse heart

Rumana Bahar, Claudia H. Hartmann, Karl A. Rodriguez, Ashley D. Denny, Rita A. Busuttil, Marijn E. T. Dollé, R. Brent Calder, Gary B. Chisholm, Brad H. Pollock, Christoph A. Klein, and Jan Vijg

Bahar R et al., Nature 2006

Article

Single-Cell Analysis of Human Pancreas Reveals Transcriptional Signatures of Aging and Somatic Mutation Patterns

Martin Engen, H. Efsun Arda, Marco Mignardi, John Beausang, Rita Bottino, Seung K. Kim, and Stephen R. Gu Kang

Enge et al., CELL 2017
Somatic mutations and disease risk
Age-related risk of breast and ovarian cancer in BRCA mutation carriers

![Graph showing age-related risk of breast and ovarian cancer with and without BRCA1 mutation. The graph indicates a higher risk with BRCA1 mutation compared to family history or no mutation.]

Ponder, Science, 1997
Somatic mutation levels in human mammary epithelial cell lines

hTERT-IMEC, BRCA1 heterozygote isogenic cell line

$P = 0.0116$

$S^*10^3$

WT

BRCA1 mutants

$n=4$

$n=4$

Sun, unpublished
Somatic mutation frequencies in primary human mammary gland cells from BRCA1/2 carriers

Primary mammary gland tissues

Median SNVs per cell per sample

(*10^3)

Control

BRCA1/2 mutation carrier

Primary mammary gland epithelial cells

SNVs per cell

(*10^3)

7 samples

8 samples

Control

BRCA1/2 mutation carrier

P=0.0385

P=0.0587

Sun, unpublished
Somatic mutation signatures in BRCA1/2 carriers and controls

Signatures are interpreted according to COSMIC database and Alexandrov et.al., 2020, Nature doi.org/10.1038/s41586-020-1943-3.

Sun, unpublished
Processing of normal lung cells for somatic mutation analysis

Bronchial brush, culture and nuclei isolation

Single nuclei collection

1. Bronchial brush
2. Culture
3. Nuclei isolation

U01 ES029519 (Vijg/Spivack; method)
U01HL145560 (Spivack/Vijg; aging)

Simon Spivack
Alex Maslov
Zhenqiu Huang
Mutation frequencies in single, bronchial cells of non-smokers as a function of age

$P = 1.62 \times 10^{-4}$

$P = 0.045$
Median single cell mutation frequencies in bronchial cells of non-smokers as a function of age

- **Median SNV per cell**
  - **Age of donor (yrs.)**
  - **$P = 1.62 \times 10^{-4}$**

- **Median INDEL per cell**
  - **Age of donors (yrs.)**
  - **$P = 0.045$**
Mutation signatures in human bronchial cells as a function of age

Signature A1 correlates significantly with the COSMIC aging signature SBS5 (https://cancer.sanger.ac.uk/cosmic/signatures; Cosine similarity 0.871)
Median mutation frequency in bronchial cells of smokers versus non-smokers across age

$P = 0.016$

$P = 0.018$
COSMIC mutation signatures in normal bronchial cells from smokers versus never-smokers
Median SNV mutation frequencies in bronchial cells from human subjects as a function of smoking pack years.
Interventions for limiting loss of genome sequence integrity

- Preventative measures, such as dietary and lifestyle changes, e.g., UV, tobacco smoke, polycyclic aromatic hydrocarbons
- Dietary antimutagens, e.g., various flavonoids, selenium, plant polyphenols
- Metabolic interventions, e.g., dietary restriction, FOXO
- Direct upregulation of DNA repair pathways
- Elimination of mutant cells
Thank you!

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Alex Maslov
Moonsook Lee
Xiao Dong
Shixiang Sun
Lei Zhang
Xiaoxiao Hao
Zhenqiu Huang
Yujue Wang
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