Topic 3: What biomarkers and stress tests predict resilience and what clues do they give us about resilience biology?

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Aging-Related Molecular Changes, Related Biomarkers, and their Utility in Resilience Detection

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Virginia Byers Kraus, MD, PhD
Duke Molecular Physiology Institute, Duke Aging Center
Duke University School of Medicine

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WHY IDENTIFY MOLECULAR FACTORS OF RESILIENCE CAPACITY?

“Molecular origins of resilience are complicated and not yet well understood”
(Ryan 2022, PMID:35599764)

01 IDENTIFY VULNERABILITIES

02 ENHANCE RISK MANAGEMENT

03 INFORM TREATMENT DECISIONS

04 IDENTIFY MECHANISMS of RESILIENCE

05 ENHANCE RESILIENCE of OLDER ADULTS

RESILIENCE CAPACITY

The capacity of a physiologic system to recover to, or improve upon, a baseline level of health and function after experiencing a significant clinical stressor (Walston 2023, PMID:37386913)

*Conceptual model of reserve and resilience.*

Resilience Capacity  
- Molecular  
- Prognostic  
(FDA: to identify the probability of a clinical event*)

*Opportunities to intervene*

MOLECULAR BIOMARKERS OF 'RESILIENCE CAPACITY'

- **GENETIC**
  - invariant DNA
  - heritability 11-50%
  - (Sawyers 2020 PMID:32108979)

- **TRANSCRIPTOMIC**
  - variant mRNA

- **EPIGENETIC**
  - variant DNA methylation
  - small RNA (micro and pi)

- **BIOLOGICAL SYSTEMS**
  - Hypothalamic-Pituitary-Adrenal Axis
  - Immune System/Inflammation
  - Sympathetic Nervous System

- **PROTEOMIC**
  - variant protein cytokines

- **METABOLOMICS/LIPIDOMIC**
  - variant human-derived microbiota-derived

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BIOMARKER “STRESS TESTS”

- Stimulus response framework analogous to a cardiac stress test
- Measured before, during and/or after a controlled stressor
- Can unmask abnormalities and endotypes: mechanistic pathways
- Examples of molecular stress tests: ACTH stimulation test; 24hr salivary cortisol profile; glucose tolerance tests; Cartilage oligomeric matrix protein (serum COMP) mechanosensitivity

Graphic provided by Dr P Jayabalans

Treadmill walking 30 mins

Biomarker response (serum COMP) in knee osteoarthritis

Higher COMP (% baseline) 3.5 and 5.5 hours after 30 min walking stressor predicted increased risk of MRI cartilage thinning at 5 years (Erhart-Hledik 2012 PMID: 22868052)
BIOMARKER “STRESS TESTS” ex vivo response to lipopolysaccharide
LPS/endotoxin is not just a “model system” it is a physiological stressor

In vivo
Natural plasma levels
(Miller 2009 PMID:18672240)
Pro-inflammatory effect
of 0.6 ng/kg LPS IV
(Mehta 2012 PMID:22709547)

LPS used as an ex vivo measure of immune response to a metabolic stressor

1. Collect peripheral whole blood
2. Dilute with RPMI (1/3-1/2 total) Add 0 or 10 ng/ml LPS (50 EU)
3. Incubate 4-24 hours at 37C
4. Centrifuge
5. Collect plasma
6. Quantify cytokine production

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BIOMARKER “STRESS TESTS” Significance of LPS in resilience

- Physiological stressor in sepsis and dysregulated metabolism/obesity/fatty liver/low level of small HDL particles (innate countermeasure of LPS) (Kraus 2022 PMID:36182774)
- Cytokine production (IL-1β, IL-1Ra, IL-10, IL-6, TNF-α) by LPS stimulation is under tight genetic control (>50% heritability) (De Craen, 2005 PMID:15674372)
- Low ex vivo LPS responsiveness (low IL-1β and IL-6) associated with the absence of Osteoarthritis (OA) in 90 year olds (Goekoop 2010 PMID:20417290)
- High responsiveness (high IL-1β and IL-1Ra, low IL-10) is associated with increased OA risk (Riyazi 2005 PMID:15880595)
- Exaggerated responses of immune system cells to LPS in frail compared to non-frail older adults (Walston 2023 PMID:37386913)
- Under chronic stress conditions, resistance develops to cortisol, another innate countermeasure of LPS (van Looveren 2020 PMID:32383538)

LPS ex vivo test system preserves physiological cellular interactions

1. Collect peripheral whole blood
2. Dilute with RPMI (1/3-1/2 total) Add 0 or 10 ng/ml LPS (50 EU)
3. Incubate 4-24 hours at 37C
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Examples of “Less is More” Resilience Biomarkers

• JAK inhibitors (immunomodulators) consistently reduce covid-19 mortality (Sweeney 2024 PMID:38182048)

• NOD/RIPK2 inflammatory signalling and OA susceptibility (Jurynec 2022 PMID:35732460)

• FKBP5 (stress response gene modulates intracellular glucocorticoid signaling) higher methylation (lower expression) in Holocaust and World Trade Center survivors (Ryan and Ryznar 2022 PMID:35599764)

Photo by Etienne Girardet on Unsplash
Study Paradigm for Resilience Capacity Analyses

1. PreOperative Deep Phenotyping

2. Total Joint Replacement

3. Outcome ERD (Expected Recovery Differential)

4. Association

5. Resilience Capacity Candidates
   - metabolic factor (aspartate/asparagine, C22, C5:1, lactate, glutamate/mine)
   - TNFR-I, miR-376a-3p, and miR-16-5p
   - explained 27% variance in ERD in Baltimore Hip Study

Parker 2020 PMID:32386291; Whitson 2021 PMID:34325481; Walston 2023 PMID:37386913
Tissues Age at Different Rates (Oh 2023 PMID: 38057571)

Choice of appropriate testing approach must consider risks to be assessed, the related tissue and biological systems under study.
What clues do molecular biomarkers give us about resilience biology?

- Resilience is a dynamic process
- Many factors underly resilience capacity
- Resilience is genetically differentiable from psychopathology
- In some cases dampened and in some cases heightened responses to a stressor are protective
- Organ-specific plasma proteins capture disease-relevant heterogeneity of aging within and across individuals that can be applied to understanding organ and organismal resilience
- New insights into aging can come from understanding molecular components of resilience capacity
Knowledge Gaps

• Does “ONE SIZE FIT ALL” or do prognostic molecular biomarkers differ by stressor, body system stressed and/or resilience outcome used?

• Can resilience capacity be strengthened?

• What treatments mitigate risk and enhance resilience capacity?

• What molecular markers mediate resilience?

• What are the limitations of the models used to identify and validate molecular biomarkers of resilience?
Research Opportunities

What are the molecular determinants of resilience?

• Establish cohorts with baseline and longitudinal biospecimens and resilience outcomes in which to discover & validate high level candidate prognostic resilience capacity biomarkers.
• Examine biomarkers in many biological systems and their roles in resilience.
• Test new resilience interventions taking into account molecular endotypes of resilience capacity.
• Examine molecular determinants of resilience capacity to different stressors.
• Adopt machine learning and artificial intelligence techniques to identify patterns and relationships that may not be apparent to human analysts.

Genetic

Epigenetic

Metabolomic

Proteomic

Graphic: adapted by Virginia Kraus from rawpixel.com