Applications of stochastic profiling

Kevin Janes
Department of Biomedical Engineering
University of Virginia

TGFB3
The breast-cancer mosaic

What role does intratumor heterogeneity play in cancer progression?

Shipitsin et al., *Cancer Cell* **11**:259 (2007)

Antonio Gaudi, Parc Güell
Heterogeneities during in vitro mammary acinar morphogenesis

Debnath and Brugge, Nat. Rev. Cancer (2005)
Identifying heterogeneously expressed FOXO targets

Sectioning

FOXO active

Akt active
FOXO inactive

e.g., GAPDH

Microdissection

Amplification
Stochastic profiling of single-cell heterogeneities in matrix-attached cells

Reference genes
Genes with measurable sampling fluctuations
Sampling CV
300%
200%
100%
0%
10-cell stochastic samplings
Single-cell coregulatory programs identified by stochastic sampling

Protein-synthesis cluster
- ALDH3B1
- FAM89B
- JUND
- MARCKS
- C14orf156
- NDUFA1
- NOLA3
- SEC61G
- RPS29
- ATP5H
- CDKN1A
- MRPL33
- RPS27L
- TBCA
- EEF2
- C17orf79
- API5
- ATP5E
- EIF3M
- RPS6
- KRT5
- C10orf116
- COX8A
- RPS21
- ILF2

Stress-response cluster
- STX8
- KIAA0101
- MRPS18C
- PRDX4
- FAM120A
- SLC25A28
- FAF2
- FKBP3
- BCL2L13
- SERP1
- FOXO1
- CCNI
- LAP3
- TCEB2
- TINP1

NFκB cluster
- BIRC3
- MAFB
- SOD2
- NFKBIA
- IL1R1

Standard deviations from geometric mean

10-cell stochastic samplings
Dichotomous nucleocytoplasmic localization of FOXO-family members

MCF10A-5E, day 10
Scale bar: 25 μm
The FOXO regulatory network

Akt

FOXO

Sesn1, Sox4, Cav1, Hdlbp, Cdkn1a, Fbxo32, Sod2, Btg1, Sema3c

- Metabolism
- Growth arrest
- Transcription
- Signaling
The FOXO regulatory network

- Transcription factor
- Transcriptional coactivator
- Unknown

**Metabolism**
- Sesn1
- Sox4
- Cav1
- Hdlbp
- Cdkn1a
- Fbxo32
- Sod2
- Btg1
- Sema3c

**Growth arrest**
- Akt
- SGK
- Cdk2
- IKK
- JNK

**Transcription**
- p53
- PR
- Ets
- SREBP-1
- C/EBP
- Sp3
- Sp1
- NF-κB
- AP-2
- GATA-6

**Signaling**
- PPARγ
- c-myc
- STAT

**Signaling**
- Akt
- SGK
- Cdk2
- IKK
- JNK
Stochastic profiling identifies two groups of FOXO-regulated genes

Stochastic profiling combined with bioinformatics identifies transcription coregulation between FOXOs and Runx1

Delayed growth arrest and altered acinar morphology in Runx1 knockdown structures

Control    shRunx1    Runx1 addback

Runx1

β-tubulin

kDa
64
49

% pRb positive acini

shGFP    shRunx1    Runx1 addback

MCF10-5E, day 14
Scale bar: 25 μm
shRunx1 phenotypes are blocked by homogenization of FOXO signaling

Vector

shRunx1

MCF10-5E, day 14

Scale bar: 25 μm

DN-FOXO1

shRunx1+DN-FOXO1

Percent pRb positive

shRunx1  DN-FOXO1

–  –  +  –  +  +

60

35

DN-FOXO1

shRunx1+DN-FOXO1

negative

DN-FOXO1

pRb

HA

β-tubulin

kDa

64

49

49

Vector:

- shRunx1
- DN-FOXO1

shRunx1+DN-FOXO1:

- Negative
FOXO-Runx1 crosstalk is blocked by anti-oxidants

A

Vector
No Trolox
shRunx1

pRb
DAPI

DN-FOXO1
shRunx1+DN-FOXO1

B

Percent pRb positive

shRunx1
- + - + +
DN-FOXO1
- - + + +

C

50 μM Trolox

shRunx1

D

Percent pRb positive

shRunx1
- + - + +
DN-FOXO1
- - + + +

FOXO-Runx1 crosstalk is blocked by anti-oxidants
RUNX1 and its understudied role in breast cancer

Kevin A. Janes
Department of Biomedical Engineering; University of Virginia; Charlottesville, VA USA

Whole-genome analysis informs breast cancer response to aromatase inhibition

Sequence analysis of mutations and translocations across breast cancer subtypes
Identifying heterogeneously regulated expression programs among ECM-attached cells

Janes et al., *Nat Methods* 7:311-7 (2010)
A pair of anticorrelated expression programs identified by stochastic profiling

10-cell samplings of matrix-attached cells

**TGFBR cluster**

~30% TGFBR3 positive

**JUND cluster**

~75% JUND positive

Standard deviations from geometric mean

MCF10A-5E d10

Scale bar: 20 μm
TGFBR3 expression represses branch-like structures during morphogenesis

A

B

C

D

MCF10A-5E d10
Scale bar: 20 μm
Constitutive JUND expression causes stable cribiform-like structures during morphogenesis.
An interlinked TGFBR3–JUND regulatory circuit in morphogenesis

---

**Figure a**

- Relative TGFBR3 levels
- Control vs. JUND-HA

**Figure b**

- Relative TGFB1 levels
- Control vs. JUND-HA

**Figure c**

- Relative JUND levels
- Control vs. JUND-HA
- Control vs. TGFBR3-HA
- Control vs. JUND-HA

**Figure d**

- Relative TGFBR3 levels
- -GDF11 vs. GDF11

**Figure e**

- TGFBR3 mRNA → TGFBR3 protein
- active TGFBR3*
- GDF11
- RFP1-Smad2
- udsVenus (P
JUND)

Positive feedback + negative feedback = oscillations?
The TGFBR3–JUND circuit gives rise to antiphase, damped oscillations.