Complex systems theory of Cancer Metastasis

Hiroshi Tanaka

Tokyo Medical and Dental University, professor emeritus

Tohoku University, Tohoku Medical Megabank Organization

special advisor to executive director

Today's Topics

Systems approach to diseases in general

- Systems (molecular) medicine, Precision medicine
- Complex systems theory to disease

☆ Complex systems approach to cancer

- **Complex systems approach of Cancer metastasis (EMT)**
  - Essential process: **EMT** epithelial-mesenchymal transition
  - **Tanaka H, Ogishima S.,** Network biology approach to epithelial-mesenchymal transition in cancer metastasis: three stage theory.

    Journal of Molecular Cell Biology, 7(3):253-66. 2015

TMDU

Introduction of myself

- Activity in JAMI (Medical Informatics)☆
  - Board of Director (21yr: 1991-2013)
  - President (2003-2007)
  - Research: Japanese version of nation-wide EHR
- Activity in JSOM (Omics-based Medicine)☆
  - President (2008-present)
  - Research: Cancer metastasis, Integration of phenotype –genotype information in cancer (iCOD database)
- Activity in CBI association (Chemo-Bioinformatics)
  - President (2011-present)
  - Computational drug discovery, drug repositioning
- Working for realization (clinical implementation) of Japan-version of Precision Medicine and Genomic Cohort

TMDU

Systems Molecular Medicine and **Complex systems theory of disease in general**

Systems (Molecular) Medicine?

System-level understanding of disease

to realize

personalized/predictive/preemptive medicine

Based on application of the concept of

"systems biology" to **"disease"**

Understanding a disease as an "**unified system**"
(interconnecting molecular network)

Tanaka H.: ***Meth. Info. Med.*** 49:173-185**, 2010**

Tanaka H.: ***Genome and Omics Medicine***
-Principle, Clinical Implementation, Big Data Approach-
a book to be appeared by **Springer in 2016**

TMDU

Systems Molecular Medicine

Views of Disease in Systems Molecular Medicine

Most of diseases, except for rare genetic diseases is not caused by aberration of one/two genes but caused by **"distortion (dysregulation) of molecular network"**

- Pathway-centric View of Diseases, due to rapid advances in knowledge of molecular pathway/network and its alternation by disease
- Inspired by success of systems biology in life science
- Other names: p4-medicine, translational systems biology

Employs disease omics profile and pathway knowledge to identify disease mechanism as caused by distorted pathways

Third Generation Molecular Medicine

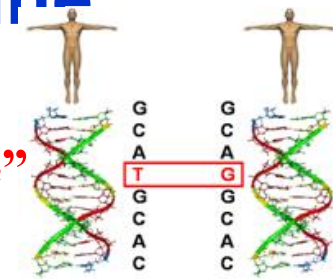
TMDU

Three generations in molecular medicine

1st generation "Genomic Medicine" (1990～)

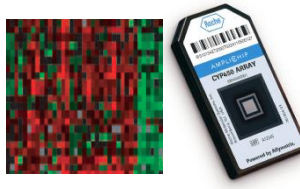
- Uses **"inborn** (germline) **personalized differences of genome"**
- Aiming at **"Personalized medicine"**

- **Estimation of "constitutional risk" of contracting disease**
  - **disease causative gene and disease susceptibility gene**
  - **Personalized medication (drug) PGx**



SNP

2nd generation "Omics-based Medicine"(2000～)


Gene expression

- Uses **"acquired somatic cell omics profile"**
- Aiming at **"Predictive/Preemptive medicine"**

Molecular systems medicine

systems pathology

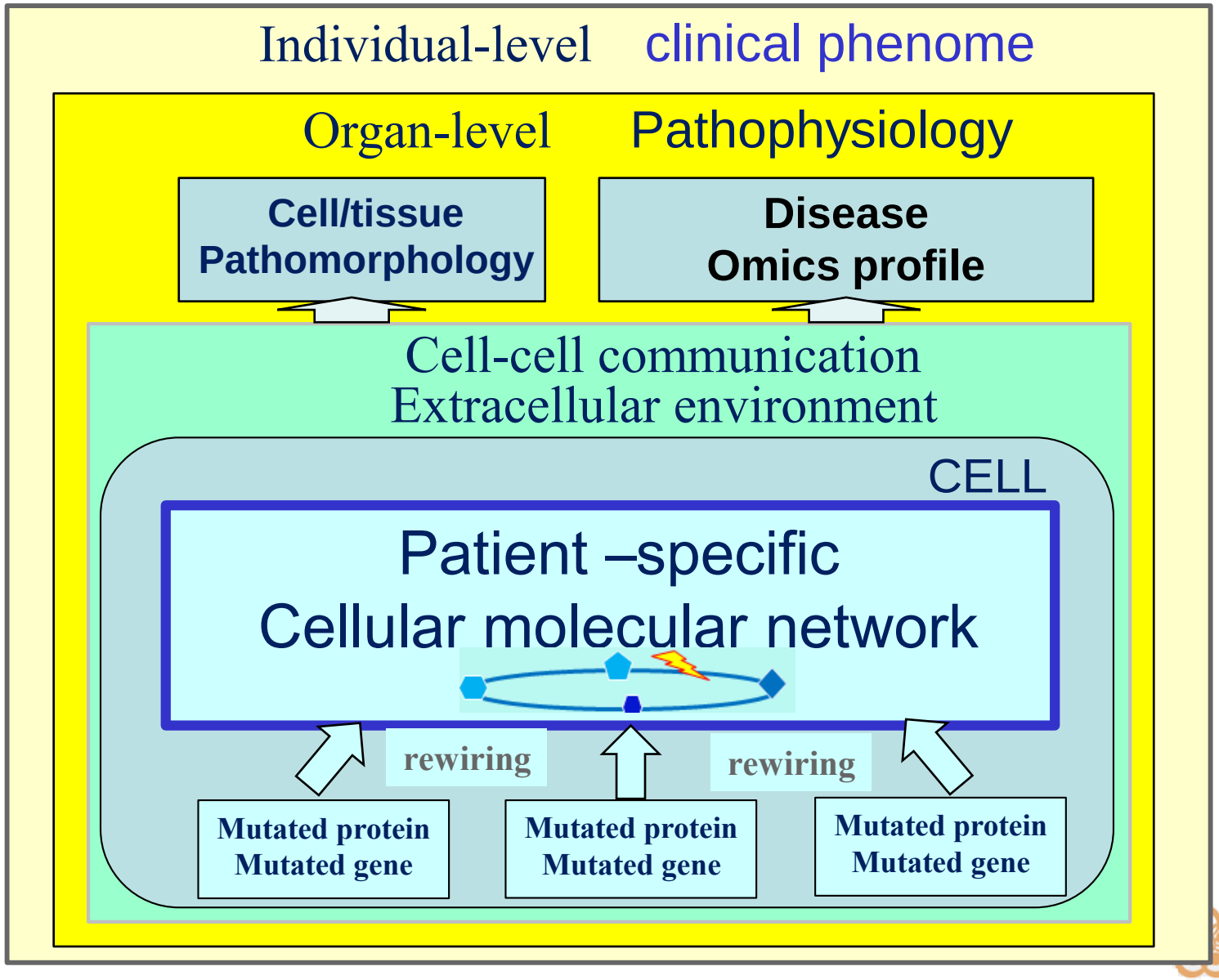
- Using **omics profile of disease** (transcriptome, etc)
  - It changes depending on disease state and tissue
- **Estimate degree of on-going state of disease progression**
  - Discover of **disease subtype** based on "omics profile", ex. breast cancer
  - Directly related to **prognosis** or **early detection** of disease (Biomarker)

3rd generation Systems molecular Medicine (2010～)

- Disease is viewed as system distortion of molecular network
- Identify **patient–specific dysregulated (distorted) pathway branches**

TMDU

Systems Molecular Medicine
Disease Model

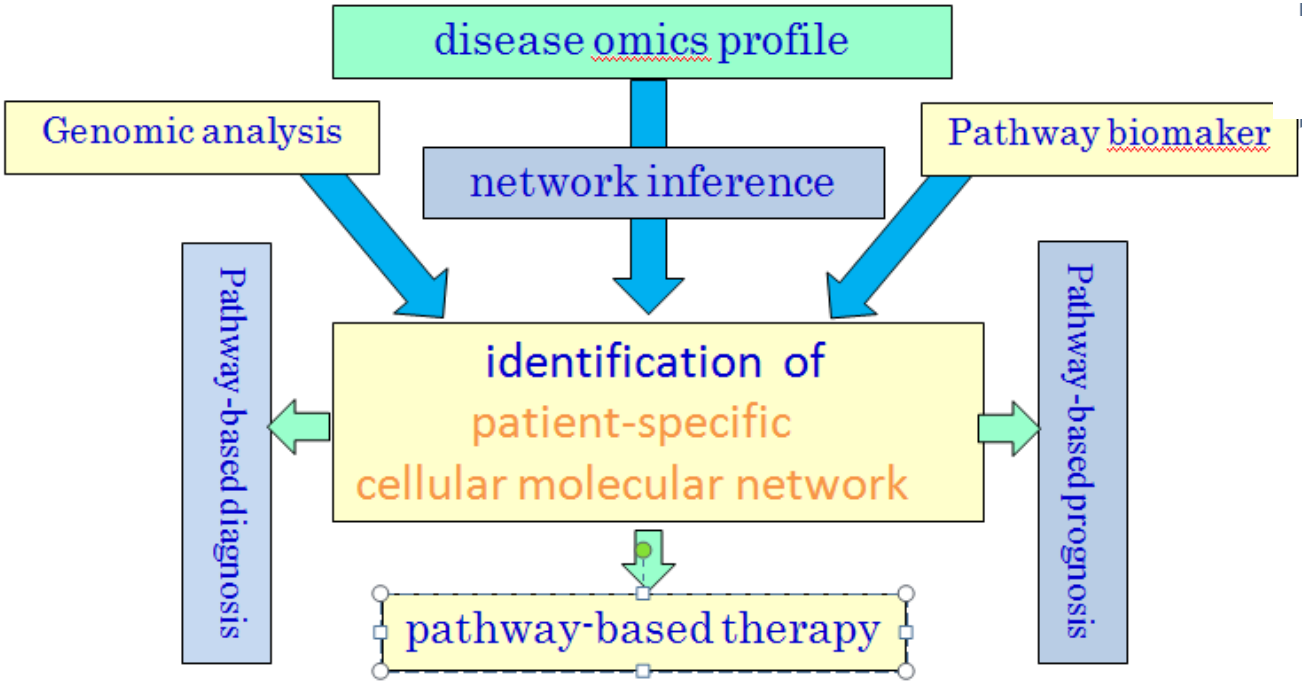


Filtered

TMDU

Basic strategy of systems molecular medicine

- Use “omics profile data” to estimate
  “patient-specific pathway dysregulation”
- Additionally use “pathway-activity-direct biomarker”, “Whole genome sequencing”
- Based on the “identified patient specific dysregulated pathway” make optimal plan of cancer treatment
- I would say **precision medicine** is **systems molecular medicine**



TMDU

Complex systems approach to disease

Complex Systems Theory of Disease

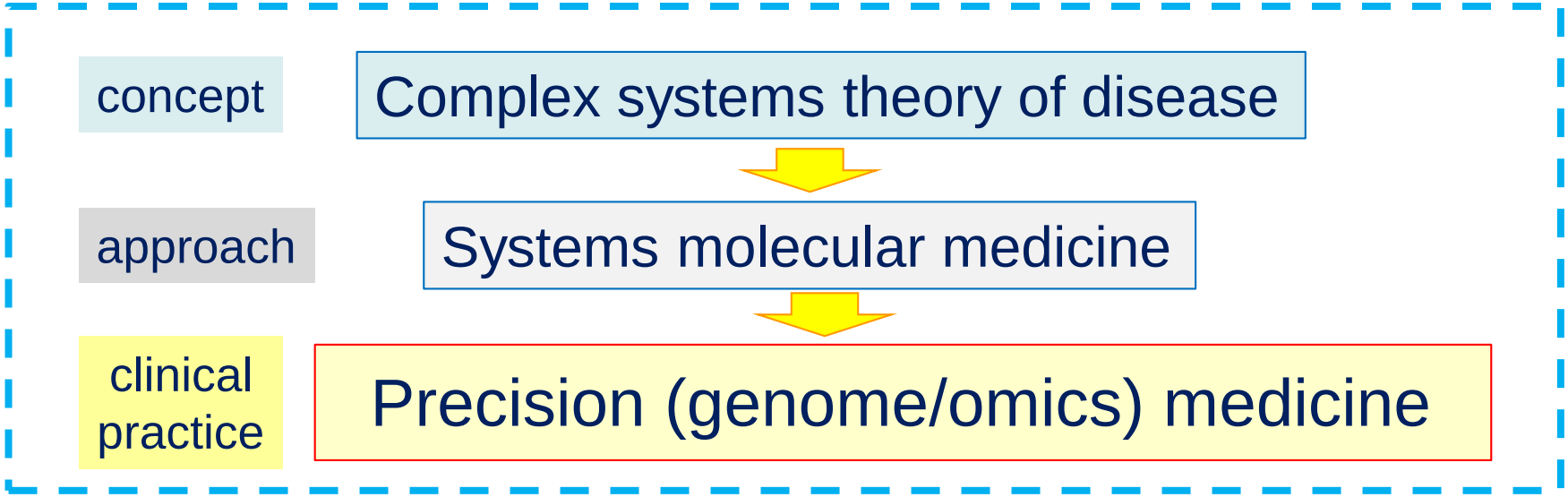
- Basis of Disease Occurrence

  ⇒ distortion of molecular network

- Progression of disease :

  ⇒structural change of molecular network

  – Trajectory in the molecular network space between quasi-potential basins in molecular network space

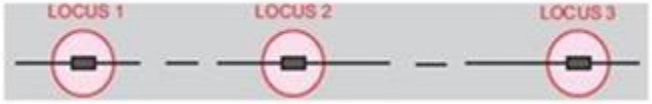


More close look at molecular network

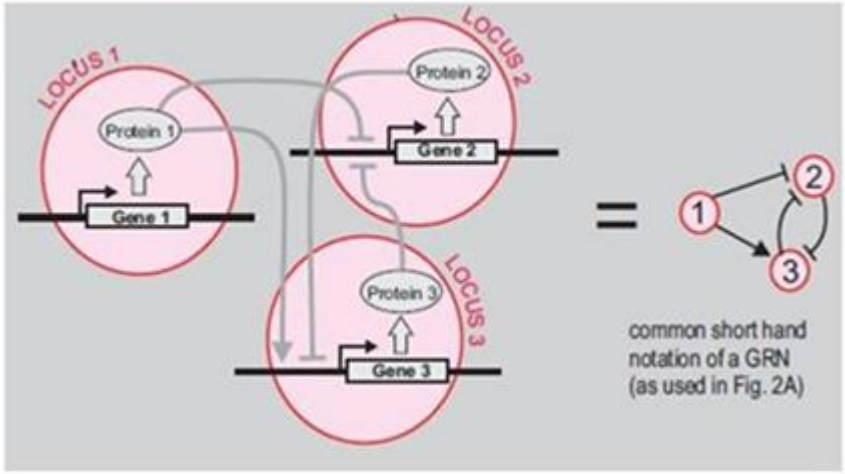
Gene regulatory network (GRN)

Gene coded in Genome

Genes are located **linearly in genome**



Innate Intrinsic Strucuture of GRN

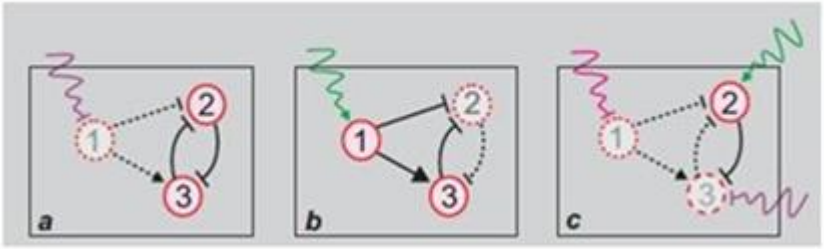


Through transcription factor,gene expression regulations are **wired innately** in constitutional **(germline) genome**.

Same basic network structure is coded in all the cells

1. All the possible sets of gene expression patterns in cellular molecular network
2. Wiring structure of cellular molecular network is innate but the set of its active nodes (proteins) and links (reactions) vary among the cell types and states
3. Equal to the space of all possible gene expression profiles

Active Subnetwork of GRN



But, actually, depending on the cell-type and subcellular/extracellular environment **a part of gene regulatory network is active**

Subnetwork of gene regulation network is active and expressed.

Huang Bioessay 34(2011)

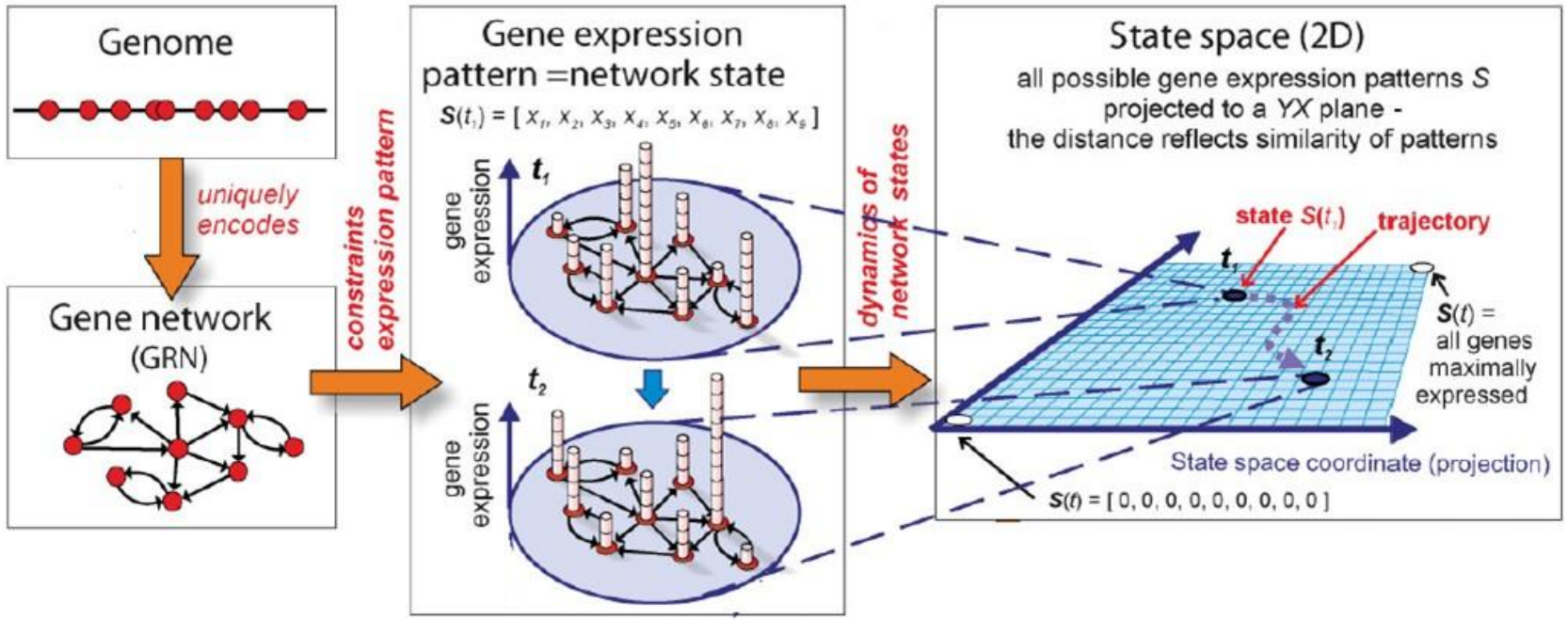
TMDU

State Space for activation of gene regulation network

“GRN space”

1. All the possible sets of gene expression patterns in cellular molecular network
2. Wiring structure of cellular molecular network is innate but the set of its active genes and links (regulations) vary among the cell types and states
3.Equal to the space of all possible gene expression profiles

GRN space comprise all possible activation patterns of gene network, as a basic framework to deal with its temporally varying activity.



Hung S. Bioessay 34(2011)

Quasi-potential in GRN space

- Quasi-potential distribution
  - the relative stability of each state in the GRN space
  - Stable state: bottom state in potential basin
  - elevation of the potential surface is inversely related to the likelihood of occurrence of the corresponding cell state
- Mathematical calculation of quasi-potential
  - Hung et.al. and Wang et. Al
  - Master eq ⇒ Stochastic Model

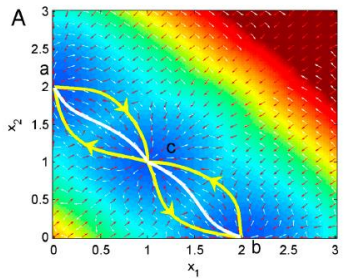
$$\frac{dx_1}{dt} = \frac{a_1 x_1^n}{S^n + x_1^n} + \frac{b_1 S^n}{S^n + x_2^n} - k_1 x_1 = F_1(x_1, x_2)$$

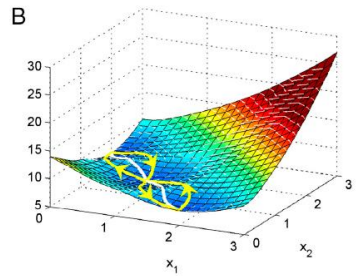
$$\frac{dx_2}{dt} = \frac{a_2 x_2^n}{S^n + x_2^n} + \frac{b_2 S^n}{S^n + x_1^n} - k_2 x_2 = F_2(x_1, x_2)$$

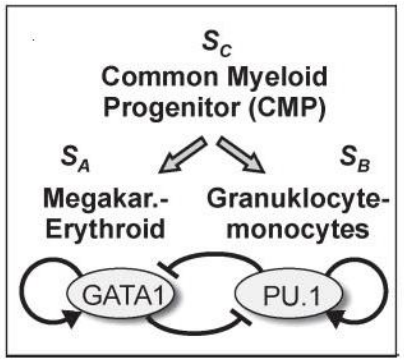
$$dx/dt = \mathbf{F}(\mathbf{x}) = [F_1(x_1, x_2), F_2(x_1, x_2)]$$

$$\frac{da}{dt} = -\lambda a$$

Wang J, Zhanga K, Xua L, Wang E, PNAS 108(20) 2011







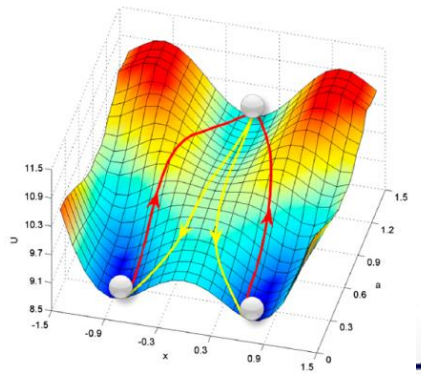


Fig. 5. The quantified Waddington developmental landscape and pathways ($a = a_1 = a_2$, $b_1 = b_2 = 1$, $k_1 = k_2 = 1$, $S = 0.5$, $n = 4$, and $\lambda = 0.01$).

Waddington Epigenetic Landscape

- Waddington proposed the **"metaphor" of cell fate**
- Cell fate : cell differentiation process

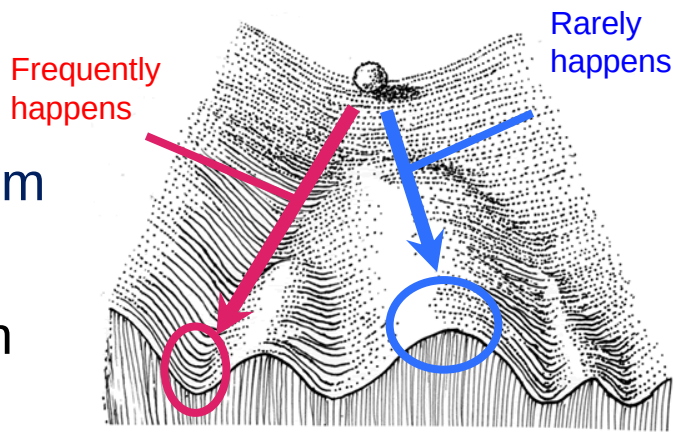
  Ball rolling down in the landscape

  from multipotent stem cell through progenitor cell to matured cell type

  Ball is rolling down along valleys separated from ridge line
- Cell choosing the one of the branched path at the foot of the ridge
- Dividing ridge:

  epigenetic gene regulation mechanism

  →"Epigenetic Barrier"
- Matured stable cell type forms the basin in the Waddinton landscape
- **GRN space equipped with quasi-potential** is thought to be "quantitative Waddington Epigenetic Landscape"



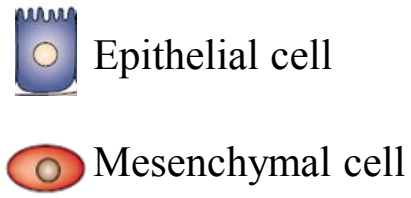
Waddington's landscape

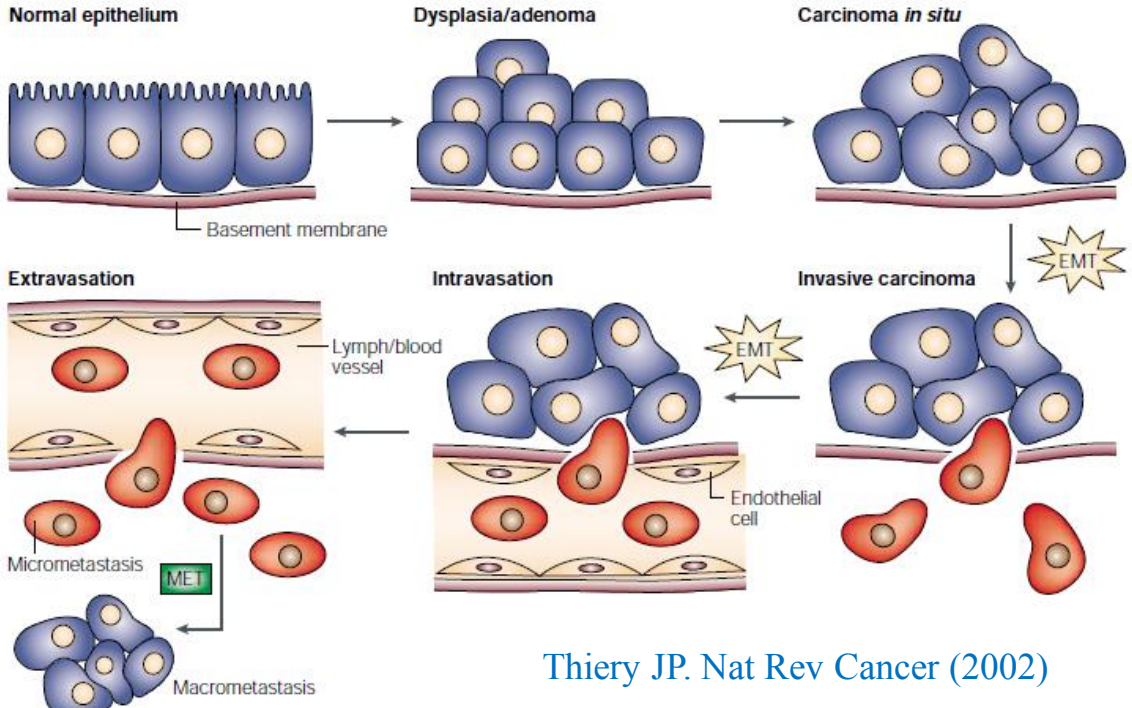
Complex Systems Approach of Cancer Metastasis

TMDU

Epithelial-mesenchymal transition (EMT)

- Primary function of EMT is a necessary step for basic developmental processes such as gastrulation or neural crest formation
- EMT is acknowdged as an essential process of cancer metastasis
  - non-motile, polarized epithelial cells, embedded via cell-cell junctions, dissolve their cell-cell junctions and convert into individual, non-polarized, motile and invasive mesenchymal cells
- EMT is a cell type conversion, hence, a structural change of gene regulation network during metastasis

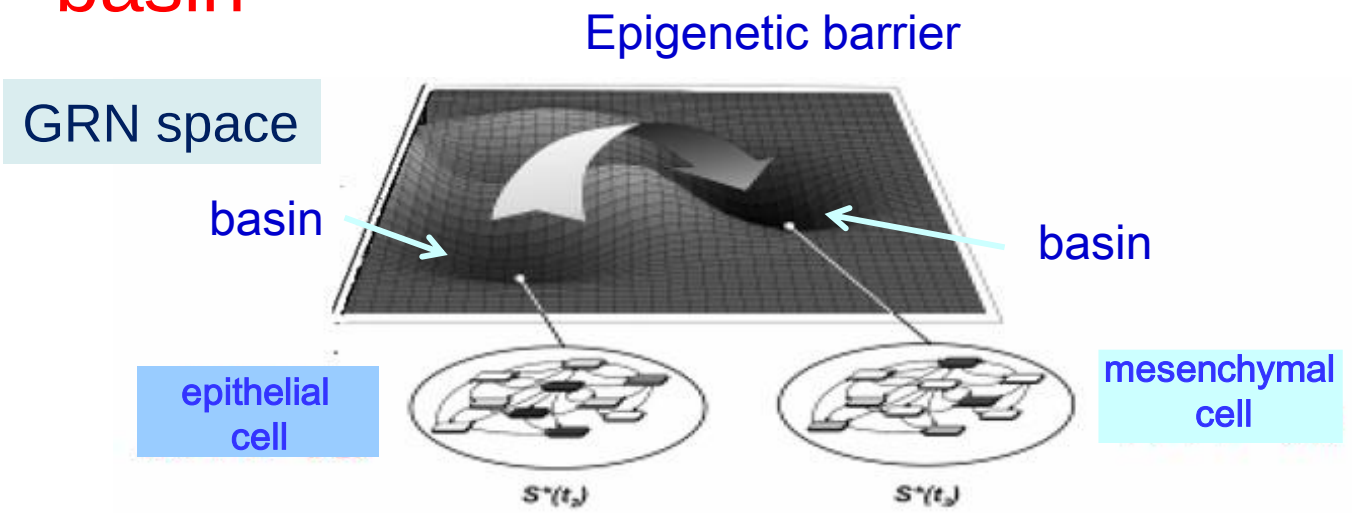




Thiery JP. Nat Rev Cancer (2002)

Trajectory of EMT process in GRN space

- EMT is structural change of GRN
- Hence, can be depicted as a trajectory traversing in GRN space
- Matured cell types stay in the bottom of basins of GRN space
- EMT is considered as transition from epithelial cell basin to mesenchymal cell basin



TMDU

Our Study on Complex systems approach to cancer metastasis: EMT

In real problem, to create the quasi-potential mathematically is vertically impossible. We developed "empirical" approach and execute "Attractor Analysis"

Article

Network biology approach to epithelial–mesenchymal transition in cancer metastasis: three stage theory

Hiroshi Tanaka[1,2,†*] and Soichi Ogishima[2,†]

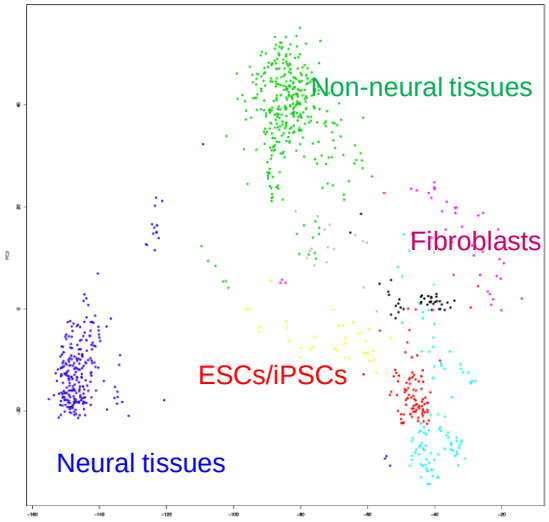
[1] Department of Bioinformatics, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan
[2] Department of Bioclinical Informatics, Tohoku Medical Megabank Organization, Tohoku University, Sendai, Japan
[†] These authors contributed equally to this work.
[*] Correspondence to: Hiroshi Tanaka, E-mail: tanaka@bioinfo.tmd.ac.jp

Journal of Molecular Cell Biology, 7(3):253-66. 2015

Our empirical method to create GRN space

(1) Create frequency distribution(φ) of cell states in GRN space

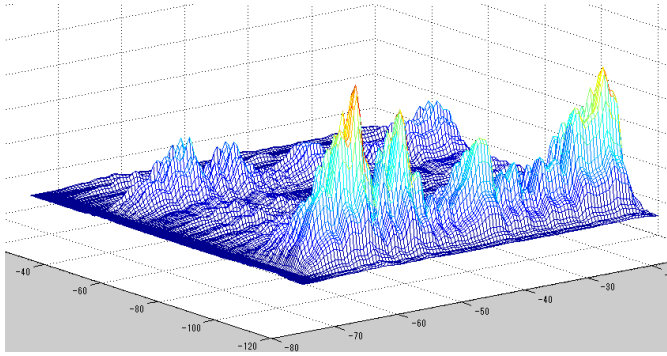
- Collect a number of samples of gene expression profiles, a particular GRN activation pattern, from
- Public DBs of gene expression profiles: GEO (Gene Expression Omnibus), ArrayExpress
- Created an empirical frequency distribution of GRN states.



GRN space

(2) Create quasi-potential distribution

- Based on the Boltzmann's principle
- The quasi-potential (ψ) is given by



(q-potential) $\psi = -\log \varphi$ (frequency)

Each cell type: commonly and frequently observed gene expression pattern

Creation of GRN subspace to depict EMT

Gene Expression Profile Data

(1) Samples of gene expression profiles of Epithelial and Mesenchymal cell types in general

- Obtained 14 datasets
- 13 datasets from GEO and 1 dataset from ArrayExpress

(2) Time course data of gene expression data on EMT process

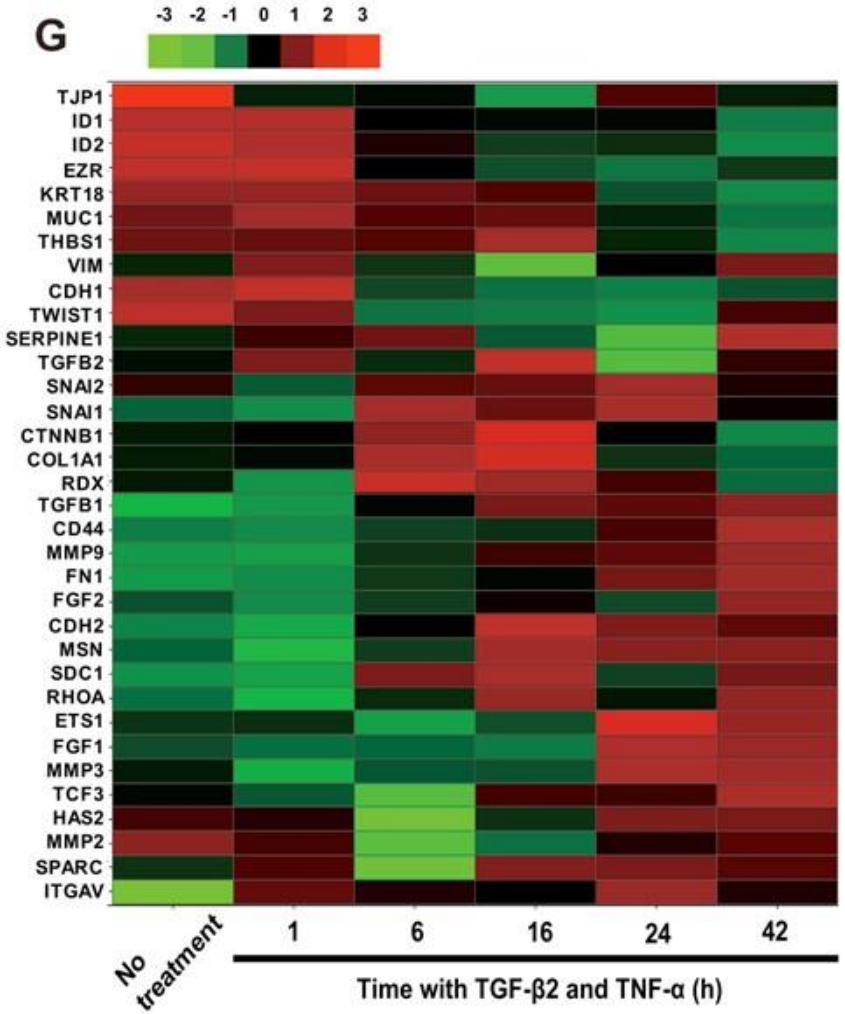
- Data source: Takahashi, E., Nagano, O., Ishimoto T., et al. (2010). J. Biol. Chem. 285(6):4060-4073.
- Human retinal pigment epithelium (RPE) cell line (ARPE-19)
- induces cancer EMT in response to the combined treatment of TGF-β and TNF-α.

TMDU

(1) Epithelial and mesenchymal gene expression data

Database	Dataset ID	Sample ID
NCBI GEO	GSE32474	GSM803648,GSM803707,GSM803765
NCBI GEO	GSE58605	GSM1414981,GSM1414982,GSM1414983
NCBI GEO	GSE50832	GSM1230054,GSM1230055,GSM1230056
NCBI GEO	GSE57083	GSM1374389,GSM1374390
NCBI GEO	GSE25146	GSM618009,GSM618010
NCBI GEO	GSE50832	GSM1230782,GSM1230783,GSM1230784,GSM1230728,GSM1230729,GSM1230730,GSM1230701,GSM1230702,GSM1230703,GSM1230045,GSM1230046,GSM1230047
NCBI GEO	GSE12548	GSM315430,GSM315431,GSM315432 (Takahashi et al. 0h hour data for epithelium)
NCBI GEO	GSE10315	GSM260657,GSM260658,GSM260659,GSM260660,GSM260661,GSM260662
EBI ArrayExpress	E-MEXP-858	31mOhC,CW31M0hA,CW31M0hB
NCBI GEO	GSE24612	GSM606843,GSM606844
NCBI GEO	GSE9440	GSM239829,GSM239830,GSM239831
NCBI GEO	GSE9832	GSM248201,GSM248202,GSM248204,GSM248209,GSM248210,GSM248213,GSM248214
NCBI GEO	GSE9865	GSM249026,GSM249027
NCBI GEO	GSE12583	GSM315617,GSM315618
NCBI GEO	GSE14897	GSM372142,GSM372144,GSM372146
NCBI GEO	GSE18226	GSM452726,GSM452730,GSM452734,GSM452735,GSM452736,GSM452737
NCBI GEO	GSE23583	GSM579884,GSM579885,GSM579886,GSM579887,GSM579888,GSM579889,GSM579916,GSM579917,GSM579918
NCBI GEO	GSE12548	GSM315446,GSM315447,GSM315448 (Takahashi et al. 60h hour data for mesenchyme)

(2) Time course data of EMT process



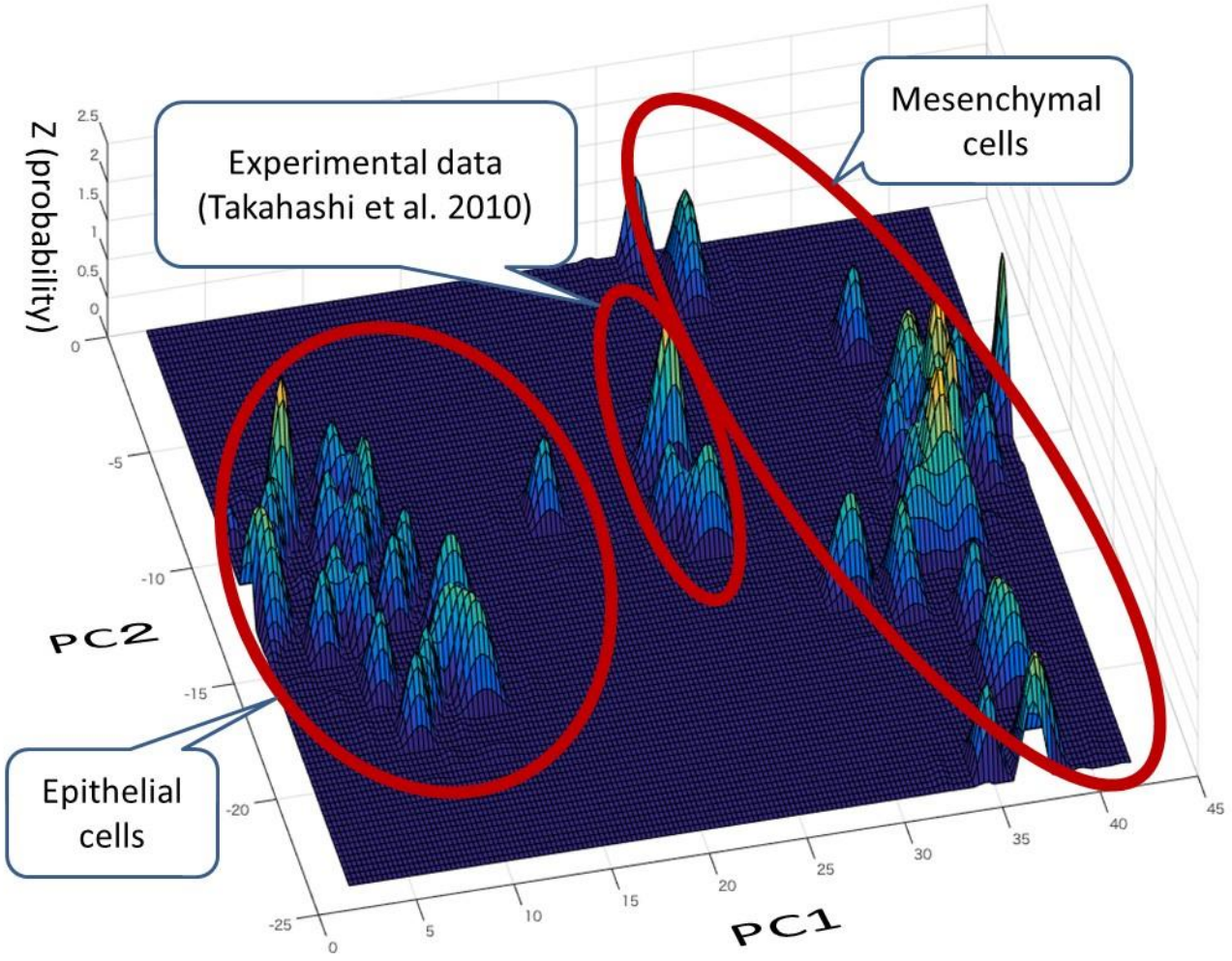
Gene expression profiles during EMT retinal pigment epithelial cell, using GeneChip (Affy HGU133 Plus 2.0).

Creation of GRN subspace to depict EMT

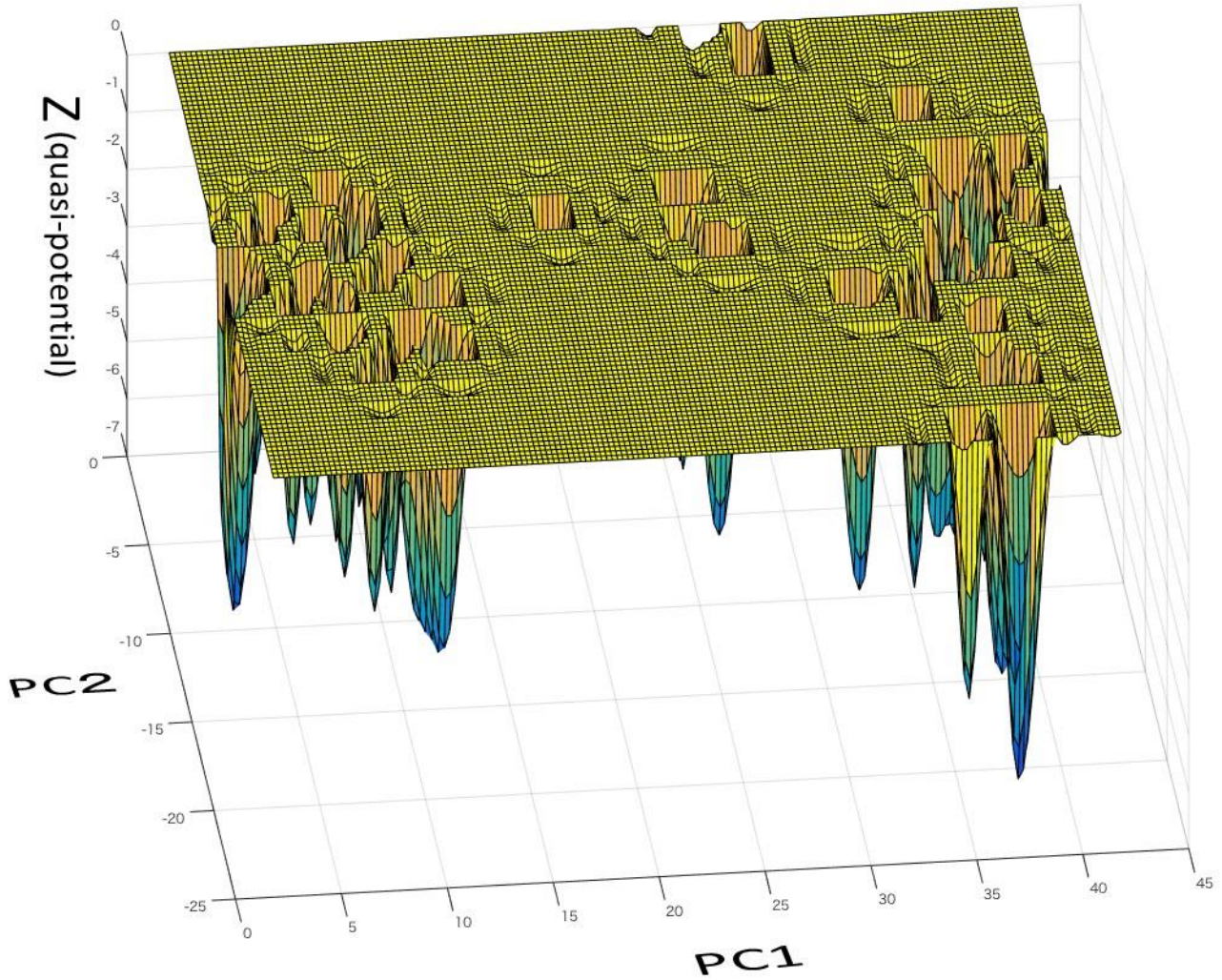
Creation of two axes for EMT depiction

- Selection of the genes for the subspace coordinates
  - 61 genes were selected
  - differ significantly between E and M cell states (significance level $p=0.1x10^{-5}$).
- Principal components used for depiction of E & M cell states and EMT process
  - applied PCA to GRN subspace of 61 genes
  - PC1 represents the general component between E and M cell states
  - PC2 is related to cancer metastasis,

Frequency distribution of E,M states and EMT process

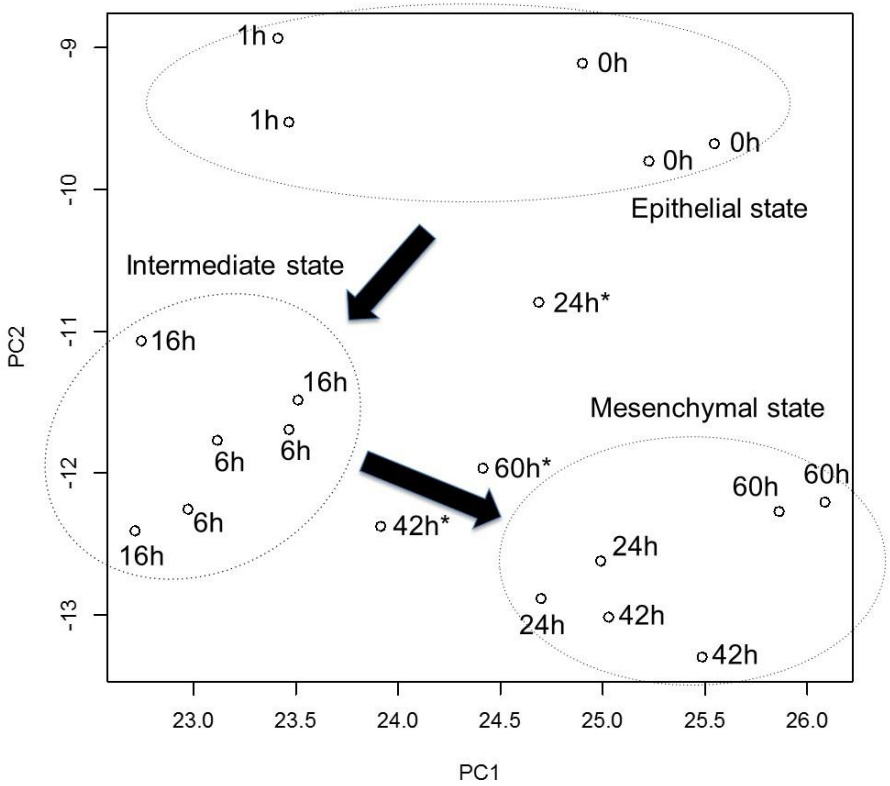


Quasi-potential distribution of E,M states and EMT process

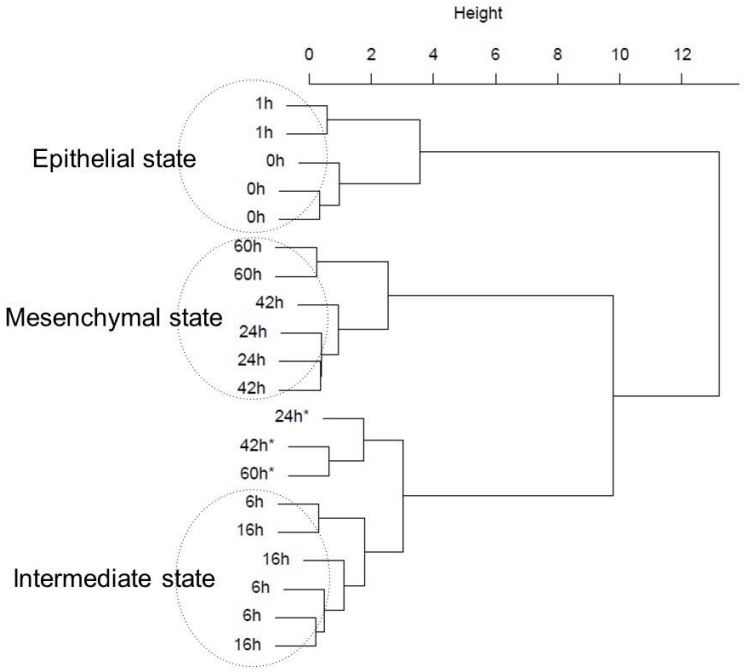


EMT trajectory in q-potential in GRN-space

Two-dimensional depiction of the trajectory of EMT



EMT trajectory in PC1-PC2 coordinates

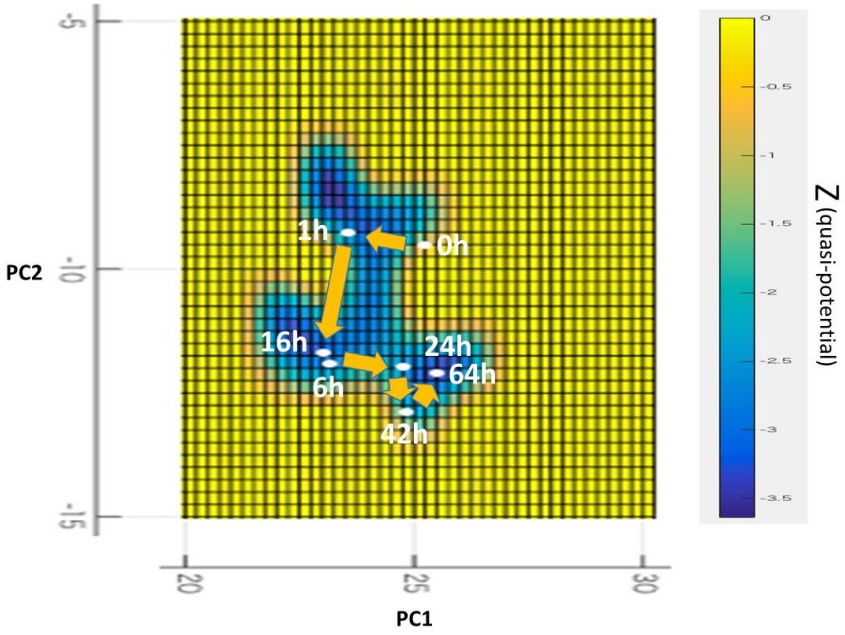


Ward's cluster analysis

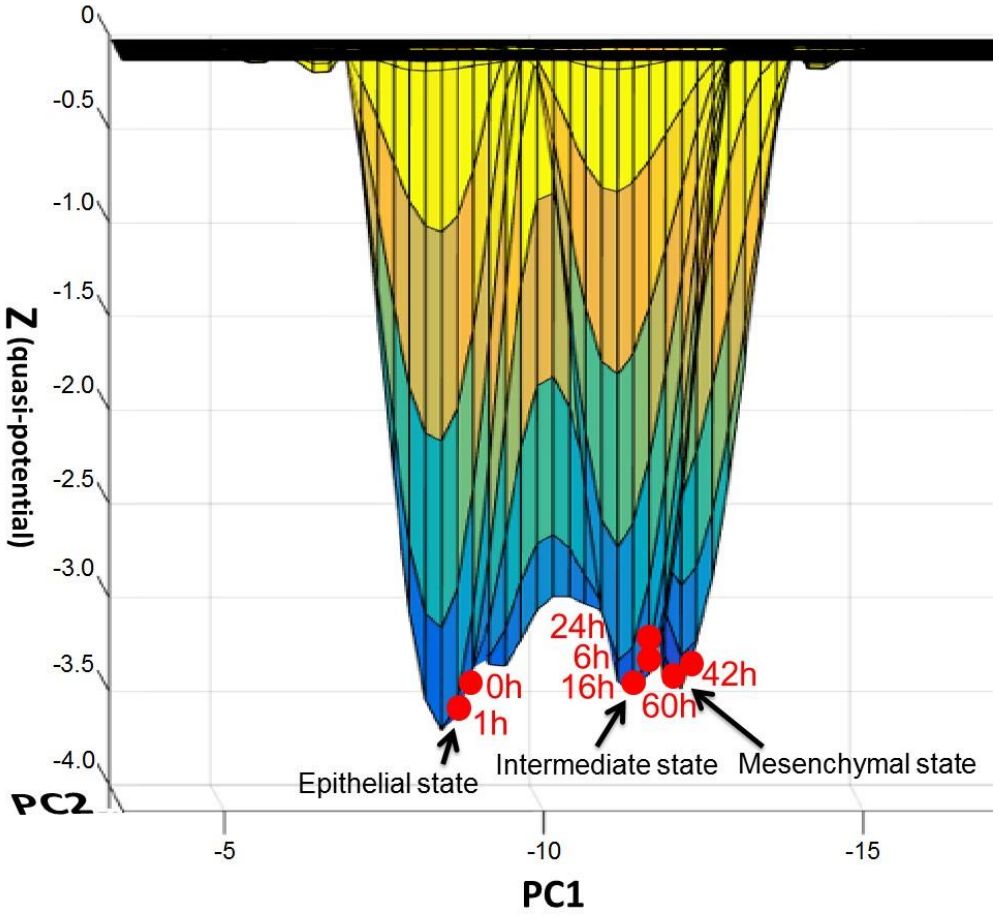
Three stages of EMT proces

EMT trajectory in q-potential in GRN-space

Three-dimensional depiction of the trajectory of EMT



Overhead view of EMT trajectory in GRN space



Lateral view of EMT trajectory in GRN space

Inference of gene regulatory network (GRN)

Selection of genes used to infer gene regulatory network

5,183 probe sets; **2,988** genes

1) Genes that showed large variations between time points during EMT; $p<0.001$; $FC>1.5$

3421 probe sets; **1776** genes

2) Genes monotonously increasing and decreasing during EMT (accumulated chi-squared test; $p<1.0\times10^{-10}$)

1,689 probe sets; **1,203** genes

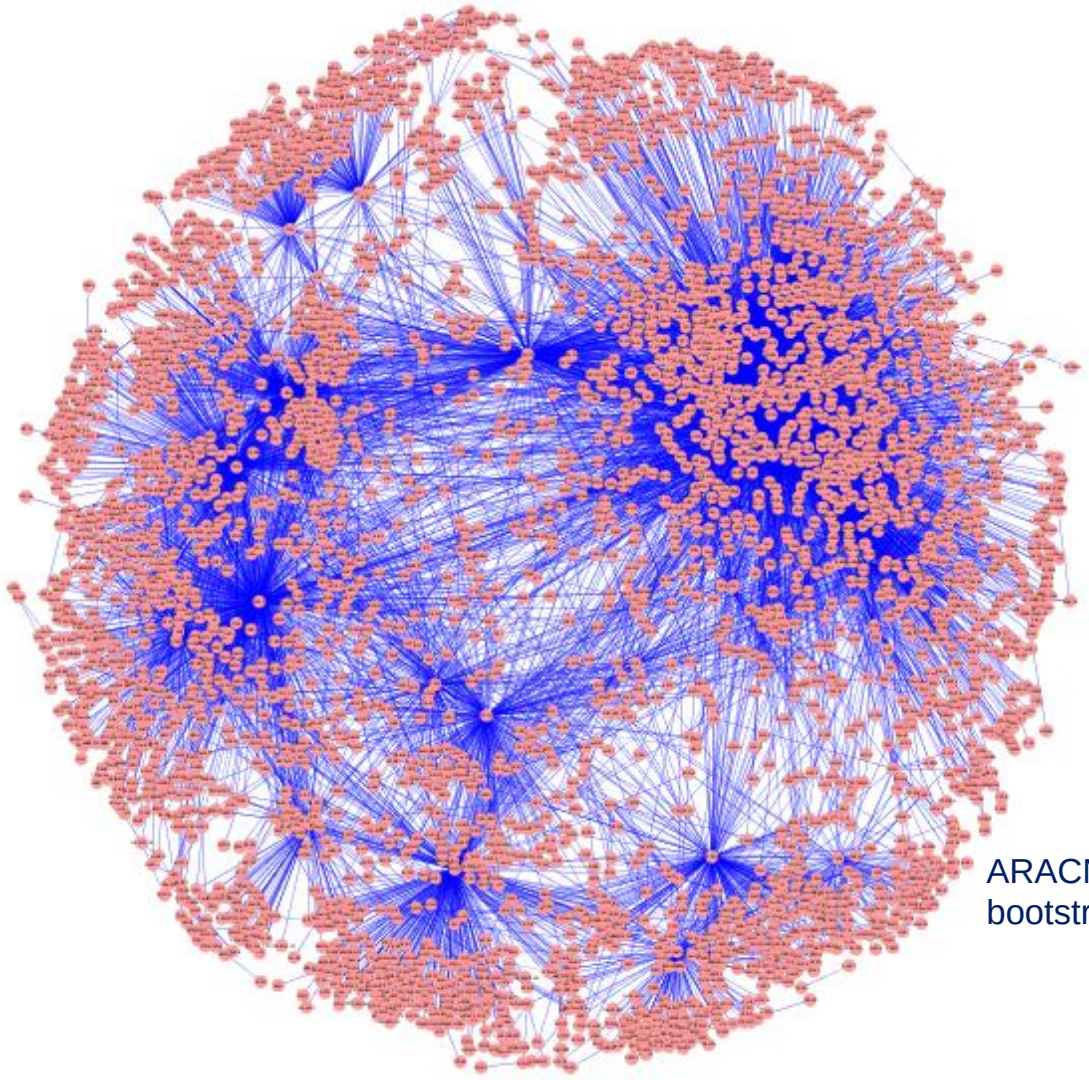
3) Collection of known epithelial/mesenchymal marker genes and EMT related genes

34 genes

TMDU

Cancer EMT gene regulatory network (GRN)

ARACNe inference of cancer EMT gene regulatory network (17,368 regulations) ($p<1.0\times10^{-10}$; bootstrap >90%).



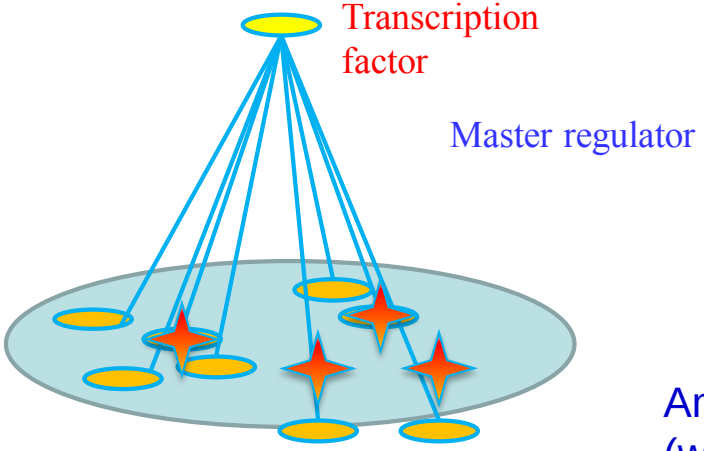
ARACNe; $p<1.0\times10^{-10}$; bootstrap >90%

TMDU

Master regulator analysis on cancer EMT GRN

Inference of master regulators which regulate more differentially expressed genes than expected (Fisher exact test p<0.05).

Cancer EMT GRN
inferred by ARACNe algorithm



transcription factors

differentially expressed genes (DEGs) (+marker genes)

genes connected to transcription factors

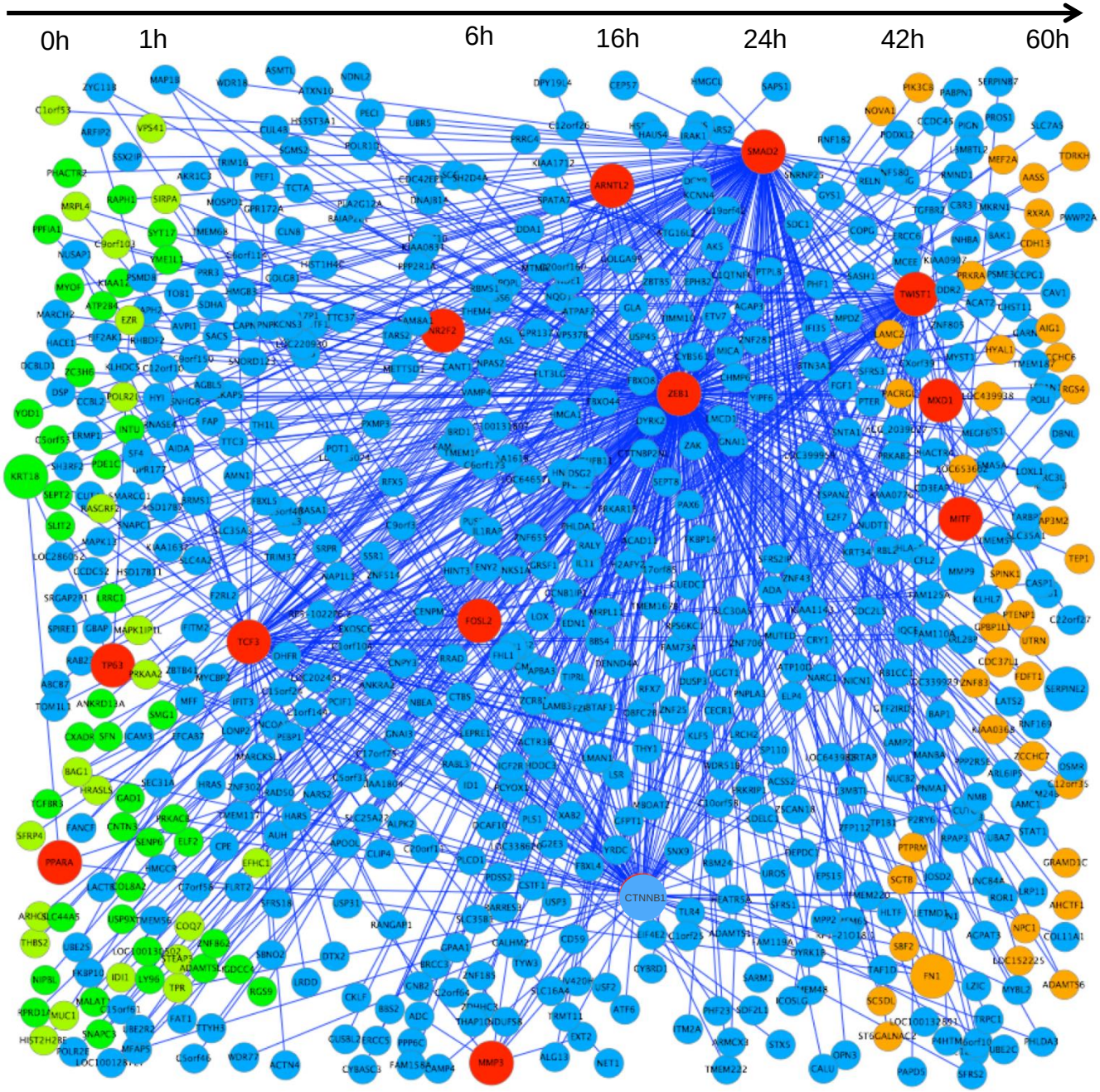
Transcription factors	# of DEG (SAM; p<0.0001, FC>1.5)	P-value (Fisher exact test)
TCF3	86/135	2.20×10^{-16}
ZEB1	46/592	8.58×10^{-16}
SMAD2	36/317	0.000209
TWIST1	16/154	0.005634
TP63	6/7	0.01302
FOSL2	5/6	0.02516
PPARA	5/7	0.03732
ARNTL2	15/38	0.03843
MXD1	4/5	0.04918
MITF	4/5	0.04918
NR2F2	4/5	0.04918

Among MRs regulating genes and their neighbor genes (within two edges in protein-protein interaction network BIOGRID), we determined EMT related genes (including epithelial and mesenchymal marker genes), and then constructed “ gene- gene network”.

TMDU

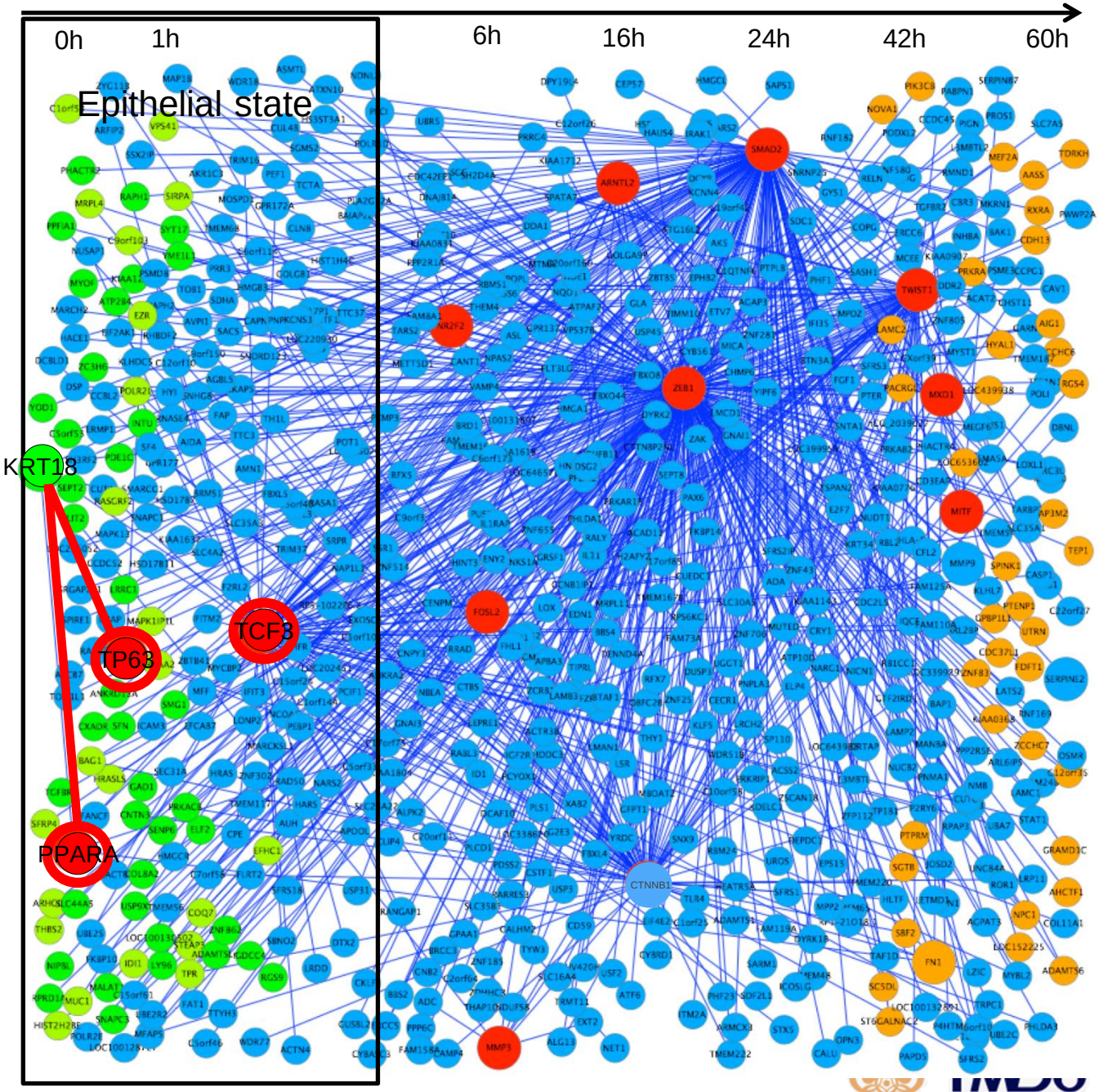
Cancer EMT network

Gene regulatory networks directly regulated by 11 master regulators.



Cancer EMT network

KRT18, TP63 and TCF3 expressed in the epithelial cells. A master regulator TP63 regulates KRT18. well-known epithelial marker



master regulator

gene regulatory interaction

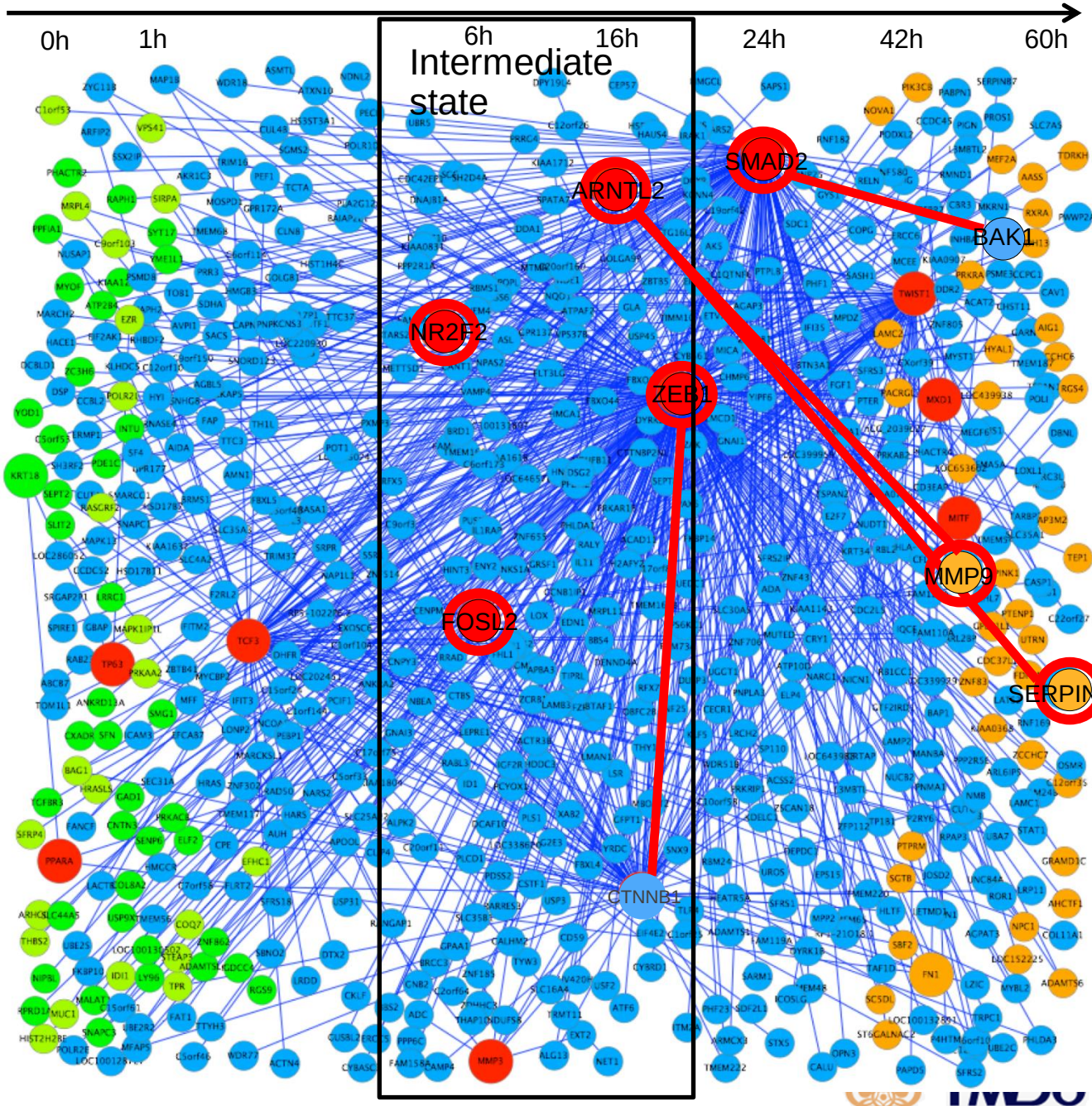
epithelial marker

mesenchymal marker

Cancer EMT network

CTNNB1 was predicted to induce the expression of ZEB1 as reported before. The CTNNB1 was known to be key factor inducing the EMT process.

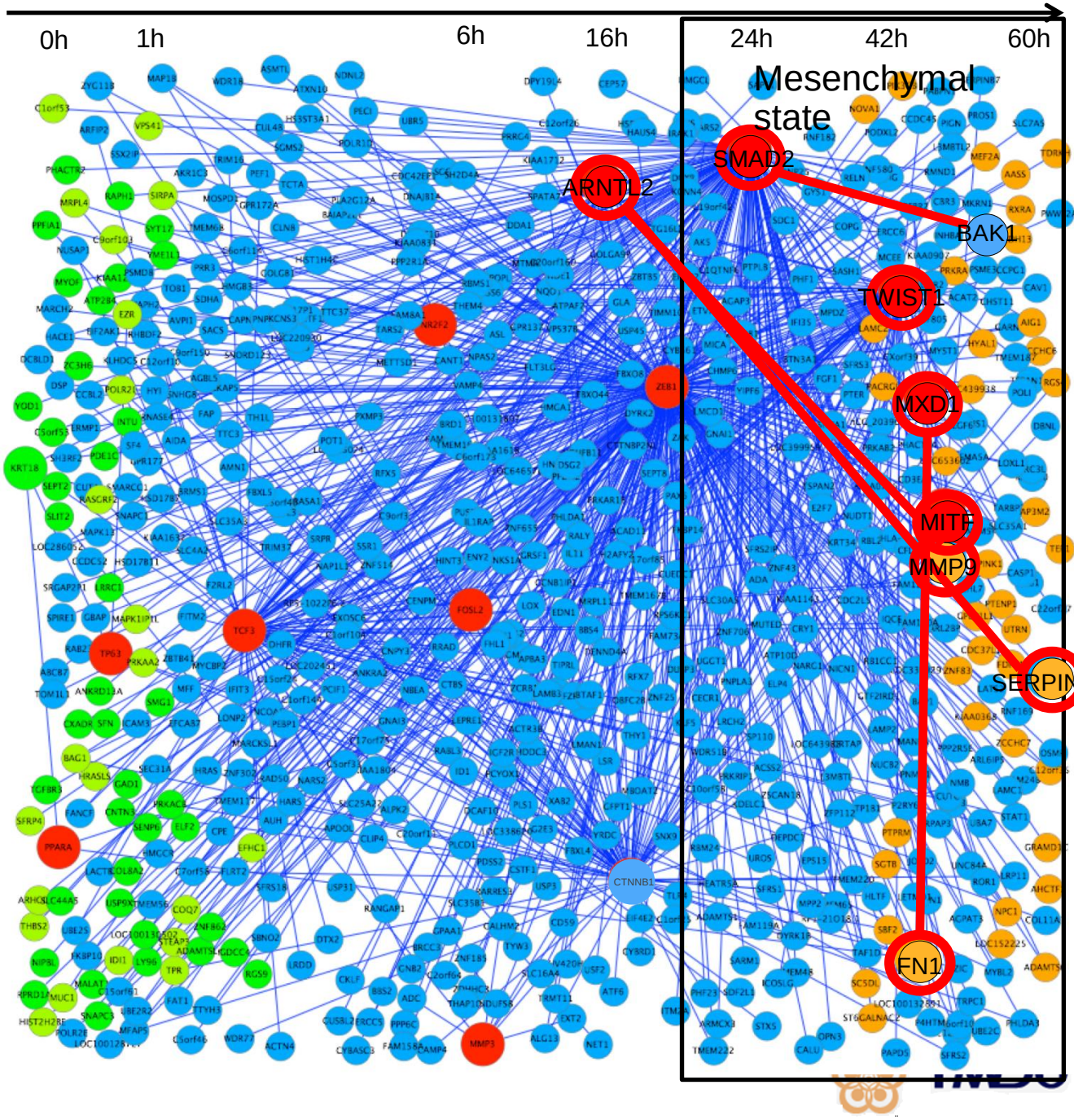
ZEB1 was also a master regulator which expressed from the beginning of EMT process, and was known to down-regulate CDH1 gene expressions.



Cancer EMT network

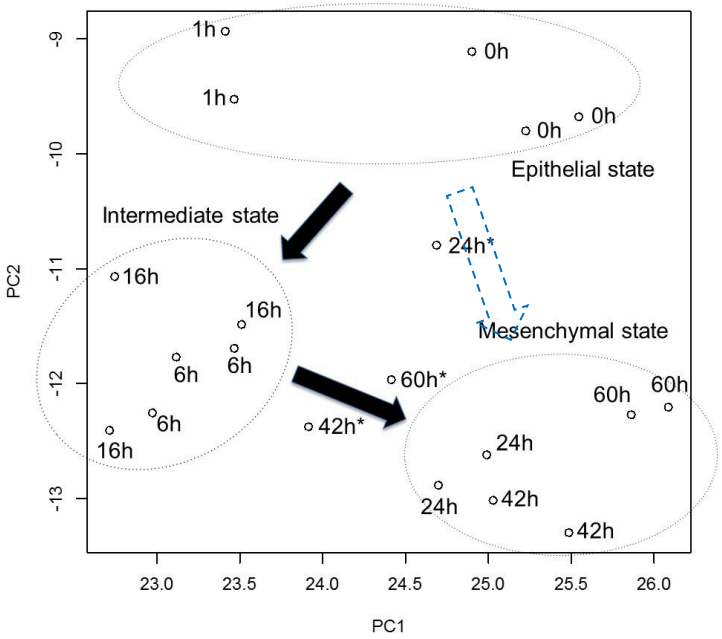
SMAD2 was a master regulator, and induce, together with ARNTL2, the mesenchymal marker genes; e,g., MMP9 and FN1, and SERPINE2 genes.

TWIST1 was also a master regulator, and in fact, was reported to be essential in induction of the EMT process.



Three Stage Theory of EMT: cancer metastasis

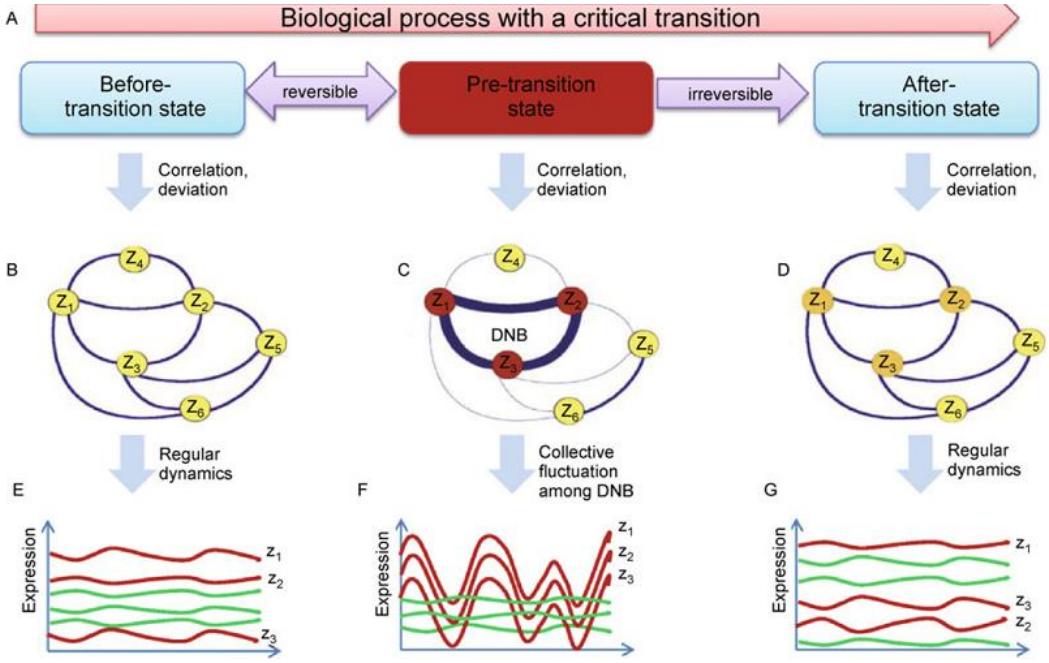
- During the process of cancer EMT, three groups of states form the separate basins
  - The first basin is epithelial and the final basin is mesenchymal
  - Trajectory shows not directly transit from epithelial to mesenchymal state
  - It makes a detour to stay at Intermediate state basin
  - Relatively stable state to form a potential basin



- Three stage theory for EMT/Cancer Metastasis
  - But what is the biological meaning of intermediate state

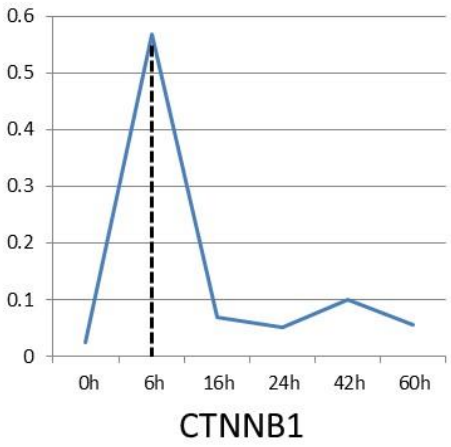
One possibility: Chen's critical transition state (DNB)

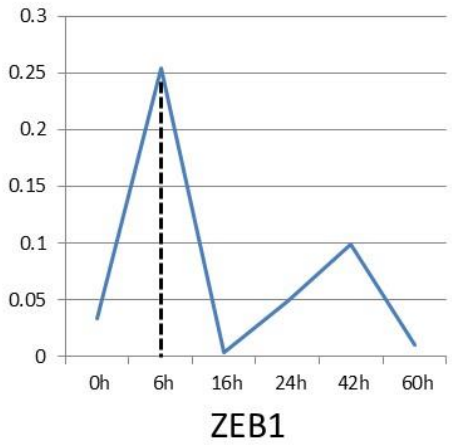
- Dynamical network theory of disease proposed by Luonan Chen
- Chen divided the disease progression process into three stages: a normal state, a pre-disease state (critical transition state), and a disease state.
- In critical transition state, strongly and dynamically correlated gene subnetwork ("dominant group of molecules") emerges
- Expression level of the members of this subnetwork increasingly fluctuates in cooperative way (DNB: Dynamical Network Biomarker)
- Other possibility: return to the undifferentiated state (stem cell state)

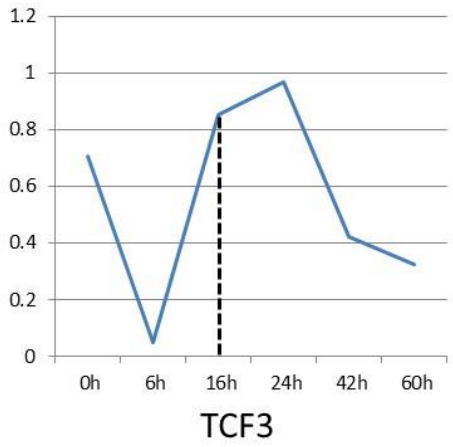


The **standard deviations** and **absolute values of correlation** coefficients among the expressions of TCF3, ZEB1 and CTNNB1

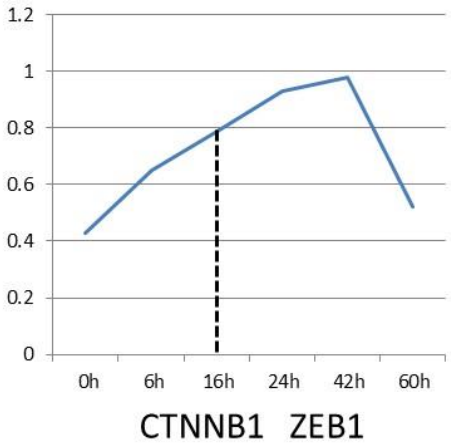
(a) Standard deviation

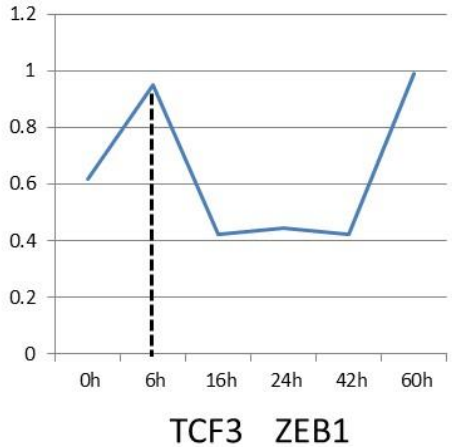


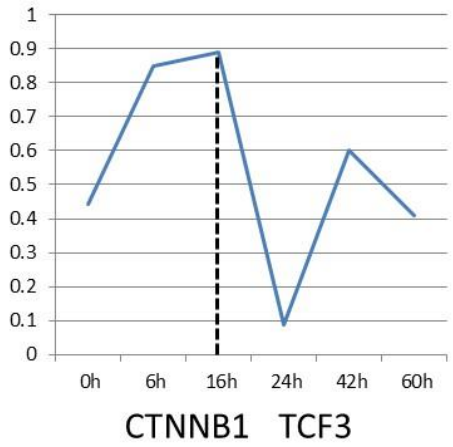




(b) Absolute value of correlation







Summary

- EMT is not just an incidental aberration of the cellular process but a biological deep structural change of the gene network, “phase transition” in physics
- Succeeded to depict the trajectory of the time course gene expression profiles of cancer EMT process onto the quasi-potential distribution in this landscape.
- 1) Cancer EMT is **three-staged process** composed of epithelial, intermediate and mesenchymal states, relatively stable state forming respective potential basin.
- 2) From the structural change in gene network, **major master regulators alternatively take a main role** along the subsequent three stages.
- The major master regulators: **TCF3 and TP63** in epithelial state, **ZEB1** with relation to **CTNNB1** in intermediate state, and **SMAD2** and **TWIST** in mesenchy state
- 3) Intermediate state of the cancer EMT is considered to correspond to Chen’s critical transition state: key molecules **TCF3, CTNNB1 and ZEB1** are highly correlated with increasing variances in the intermediate state, corresponding to the specific features of Chen’s critical transition state.

TMDU

Future Work

- To establish the three stage theory of cancer metastasis
  - More detailed observation of “intermediate states: (6h,16h) ⇒(4,6,8,10,12,14,16h)
  - To determine “stem-cell like state” or “critical transition state”
- To develop the diagnostic method for intermediate state
- To develop the strategy for drug discovery targeting the intermediate state; anti-metastatic drug

TMDU

Thank you for kind attention