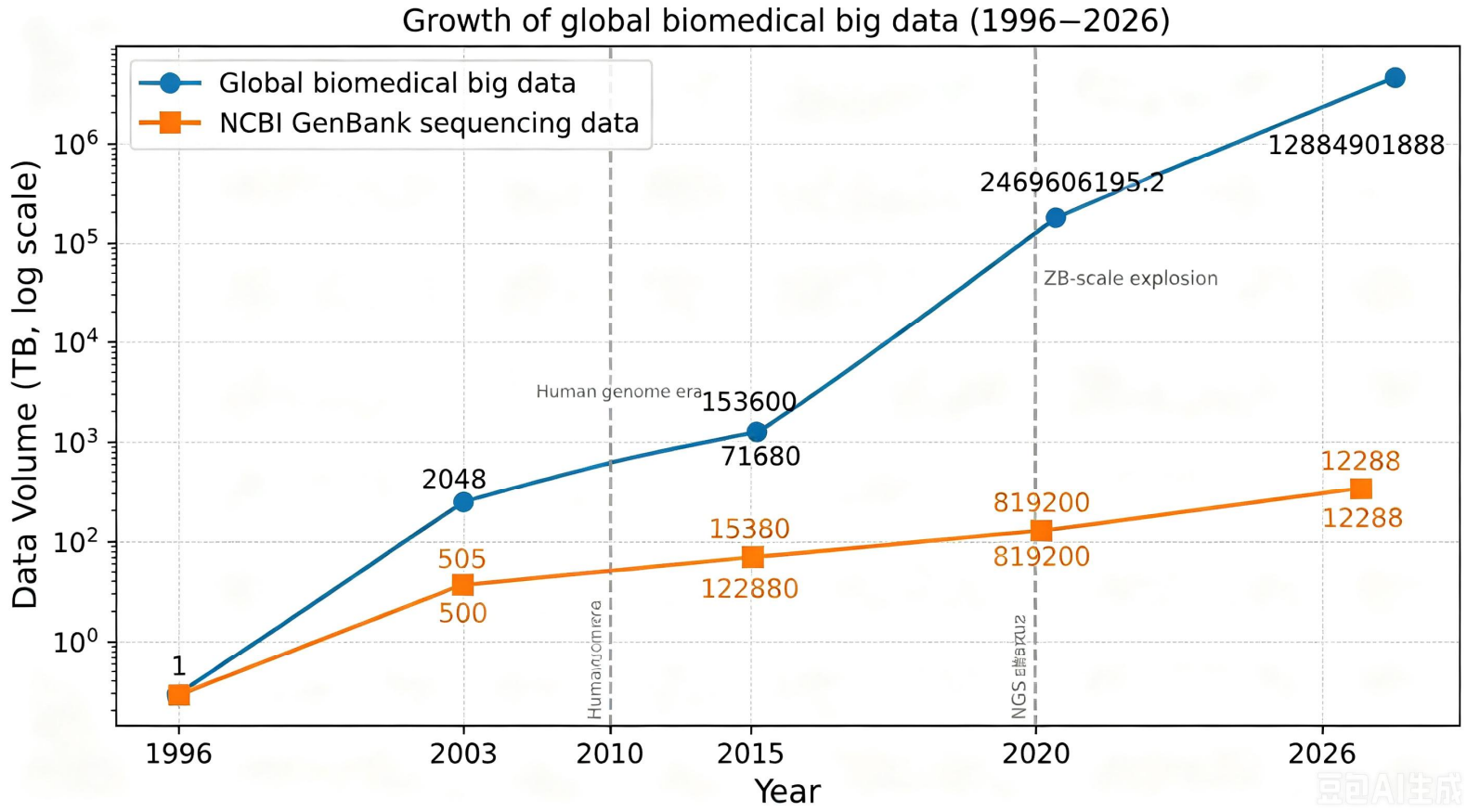
Lecture II

A Model for Cancer Evolution:

Study of cancer from the perspective of chemical in-homeostasis

Table of Content

生物医学组学大数据：30年增长了一百亿倍



Technological Advances and Over-Optimistic Views

- On June 26, 2000, US President Bill Clinton claimed: **A revolution in preventing, diagnosing, treating, and curing disease** when announcing completion of the first human genome sequencing project

- Genomic medicine holds the ultimate promise of **revolutionizing** the diagnosis and treatment of many illnesses.

  - Frances Colins and Victor A. McKusick, JAMA, 2001

- We can **cure all diseases** with the help of AI, within the **next decade or s**

  - Demis Hassabis, CBS, 2025

Reality Check: Other Major Diseases

Major revolutions in medicine as we were promised, are yet to happen with the enormous amounts of omic data accumulated and the revolutionary technology developments

Rapid Progress in Normal Biology Research

- Significant progress has been made in our understanding **of biology under physiological conditions** in the past 50-60 years
- We now understand some of the most complex systems in our bodies, e.g.,
  - the **adaptive immune system** of vertebrates
  - the **regulatory network** of embryonic development
  - the entire human single-cell homeostasis network, comprising tens of thousands of different cell types, regulated by the **endocrine**, **nervous**, **immune**, and **metabolic systems**

The Key: Conserved Biological Structures and Functions

- **A key reason**: High levels of conserved biological structures and functions exist across different organisms, even among evolutionarily distant organisms
- Utilizing this conserved nature among structures and functions, scientists have gained great amount of knowledge about human biology through studies of simpler **model organisms** quickly
  - Knowledge of the **human metabolic system** largely came from studies of **yeast**
  - Knowledge of the **human neural system** largely came from studies of **C. elegans**
  - Knowledge of the **human developmental system** largely came from studies of **Drosophila**

为什么我们还远没有达到我们的目标：

Disease Biology Is Different from Normal Biology

- A **fundamental** reason for the successful application of this approach is that cells of model organisms share highly **similar physicochemical microenvironments** with the relevant human cells.
- However, this similar physicochemical condition **does not exist** between **chronic disease cells** and **healthy cells**
- This makes it difficult to use **model organisms** to obtain large amounts of information for disease research, e.g.,
  - All mouse cancers can be cured, but the corresponding treatments have limited effects on human cancers.

Disease vs. Normal Biology

- Disease biology is essentially **stress-response biology** in a sustained manner
- **Characteristic 1**: majority of the regulation is done through **epigenomic changes** or **genomic mutations**, rather than **regulation programs** encoded in the genome
- **Characteristic 2**: considerable reprogrammed metabolisms (**RMs**) take place; these RMs have not been optimized via long-term evolution, hence may create abnormal behaviors
- **How should we study disease biology?**
  - Back to the basics

It Is Hard To Be a Cancer But Why?

- Cancer tissue cells consume up-to 20 to 30-fold more glucose than normal matching cells
- They require significantly more ATPs than normal cells
- Their levels of stresses, e.g., ER stress, are significantly higher than the normal cells
- Over 97% of cancer cells die soon after they are created
- Hence, energetically it does not seem to make sense for normal human cells to take the route to become cancerous.
- Question: why do they still do it?

A Major Hypothesis

- All such cells are under the same yet-to-identify stressors that they must take the cancerous route since otherwise they will die 100%?

生物演化的法则

- 关于微生物的演化，学者们早已建立了以下广为微生物学者接受的演化框架（在压力下，适者生存）：

> 生存压力 → 基因突变/表观改变 → 有些被选择来启动代谢重编程以适应压力

- 这些压力可能包括物种之间的竞争，微环境的改变等
- 同样地，这一框架也被广泛用于研究植物的演化，如植物如何适应土壤的干旱、水涝、虫害等
- 但人类疾病学者似乎不愿意接受这一观点，认为“基因突变”是肿瘤发生的终极原因

Red Queen Hypothesis: an example

- Paterson *et al.* designed two experiments to study coevolution of the bacterium *Pseudomonas* and its antagonistic phage Φ2
  - Have them evolve to a dynamic equilibrium
  - Then give one a slight competitive edge and keep the other unchanged
- The finding is that the equilibrium shifts towards increased population of the organism with an advantage, leading to the evolution of the other one till the original equilibrium is regained
- They concluded that antagonistic coevolution is a cause of rapid and divergent evolution, and is likely to be a major driver of evolutionary change

Paterson et al. Nature 2010

如果我们应用这一框架来研究肿瘤

- 什么是驱动肿瘤发生、发展的生存压力？
- 压力可能较难直接研究，那就研究肿瘤中保守的代谢重编程
- 肿瘤喜欢使用从头合成的方式产生核苷酸，尽管细胞可从血液中回收核苷（加磷酸化）。我们发现：越恶性的肿瘤，从头合成核苷酸的比例越高
- 肿瘤中的氨基酸代谢高度活跃，但释放氨基酸代谢废物氨气的尿素循环在所有（TCGA）肿瘤中都被抑制
- 肿瘤使用低效的发酵方式，而不是正常的、更高效的呼吸链产生ATP（瓦博格效应）

肿瘤研究的信息来源

- 我们系统地分析了**TCGA数据库**中的33种肿瘤，～2万个肿瘤组织的转录数据 – 这是世界上最大的肿瘤组织数据库、以及**GEO数据库**、**GTEx健康组织数据库**
- 从每个肿瘤组织，获取～65000个基因的表达数据，其反映了一个基因的活跃程度 – 25000 蛋白基因 + 40000 RNA基因
- 一个基因在肿瘤中的表达值比对应的正常组织中的值高，就说这个基因上调了；如果低，就下调了
- 我们特别关心与pH有关的每个酶促化学反应

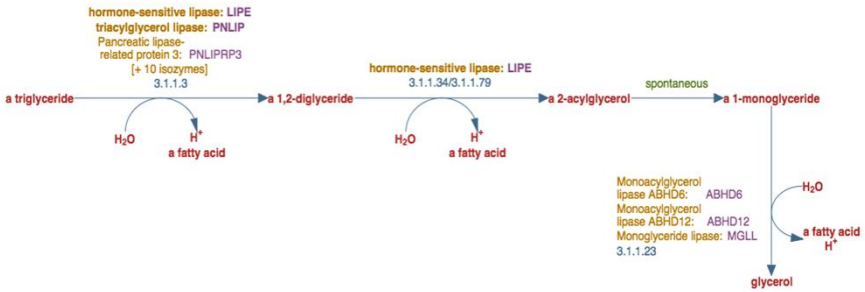
$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$$

P450酶催化的反应

这类信息来自**BioCyc数据库**

代谢重编程：核心技术

- 代谢通路：细胞将食物转化为能量、生物分子（DNA、RNA、蛋白、多糖、脂肪、有机酸等）（或反之）、及排出废物的化学反应过程，多由酶促反应来实现
- 这些通路都经过长期演化的选择、优化。一个通路的全部基因可通过某种调控方式被共同启动来完成一个将化合物A 转化成B的过程



- 代谢重编程：在持续压力下，细胞能通过某些机制组装新的代谢通路，选择其中的某些来帮助细胞适应压力

- 肿瘤中的代谢重编程

丝氨酸从头合成

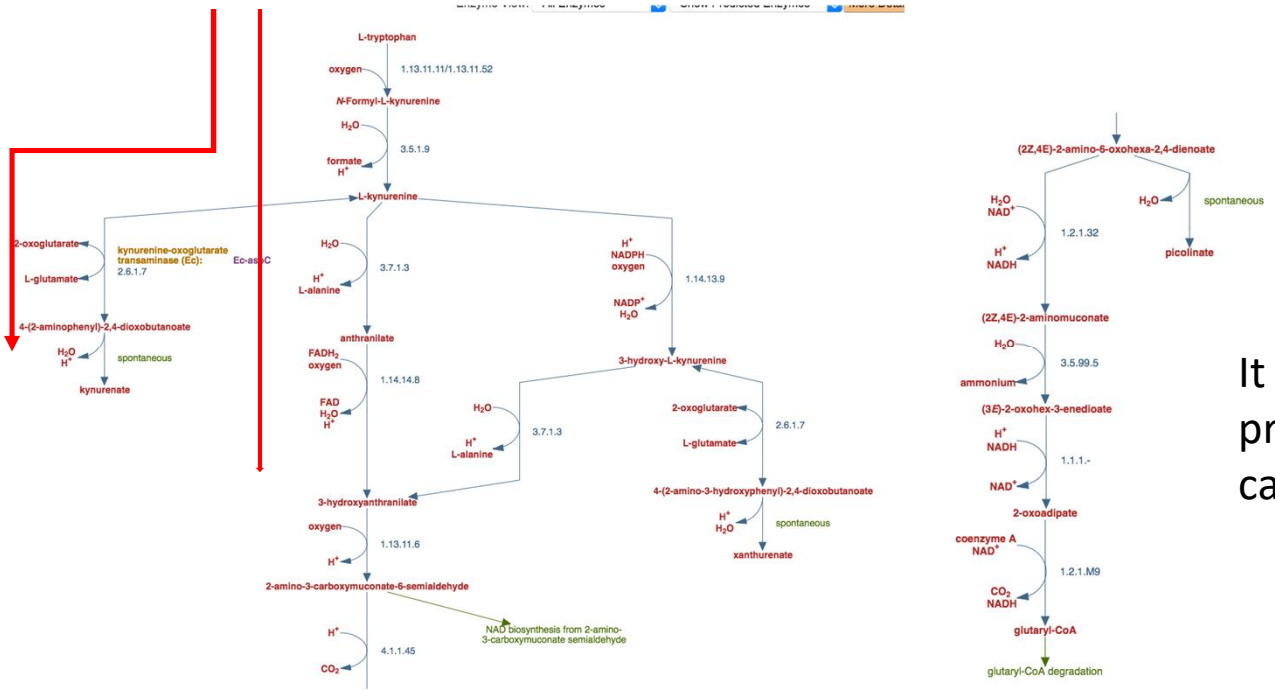
- 肿瘤生长需要丝氨酸，本可从血液中摄取，但肿瘤更倾向于自己从头合成；而且肿瘤的恶性程度越高，自己合成的比例越高

$$\text{glutamate} + 3PG + NAD^+ + H_2O \rightarrow 2\text{-oxoglutarate} + P_i + \text{serine} + NADH + H^+$$

- 从头合成比从血液中摄取多产生一个H^+

Tryptophan Degradation in Cancer

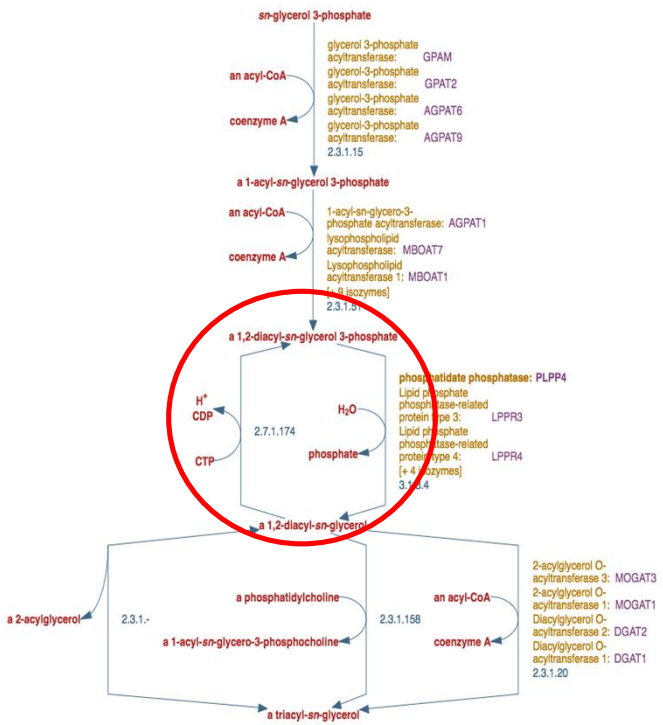
- Normal cells degrade tryptophan to acetyl-CoA via the following pathway but cancer only uses part of the pathway to produce kynurenine and 3-hydroxyanthrranliate



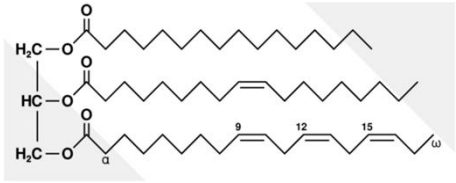
It produces most number of protons and the end-product can be released

甘油三酯的合成及降解

- 有些肿瘤合成甘油三酯、然后就降解它。为什么？

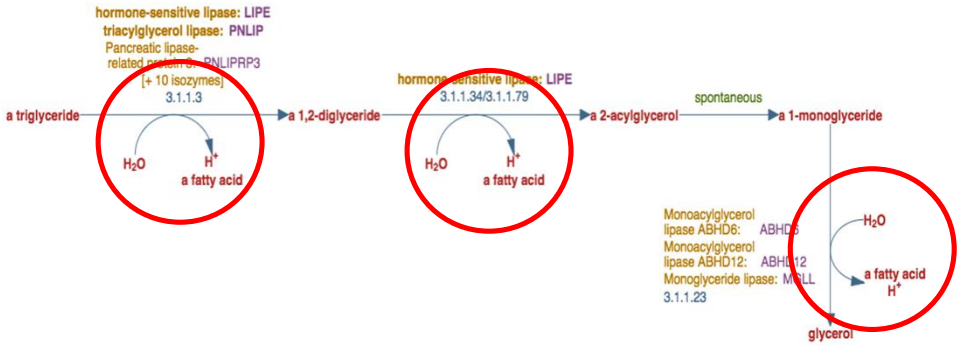


合成一个甘油三酯
会同时产生多个H^+



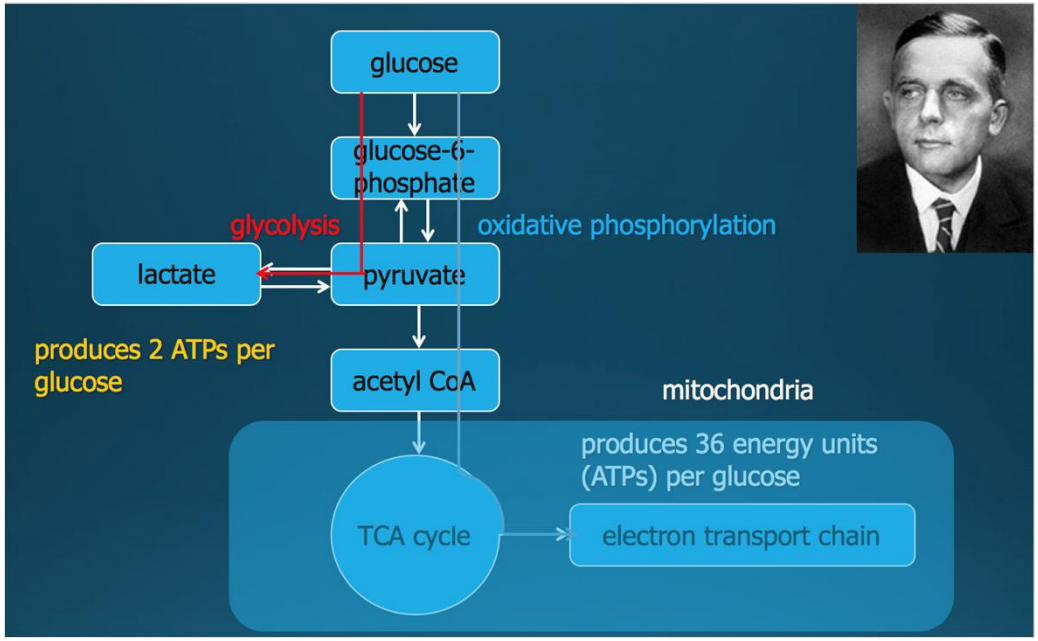
甘油三酯

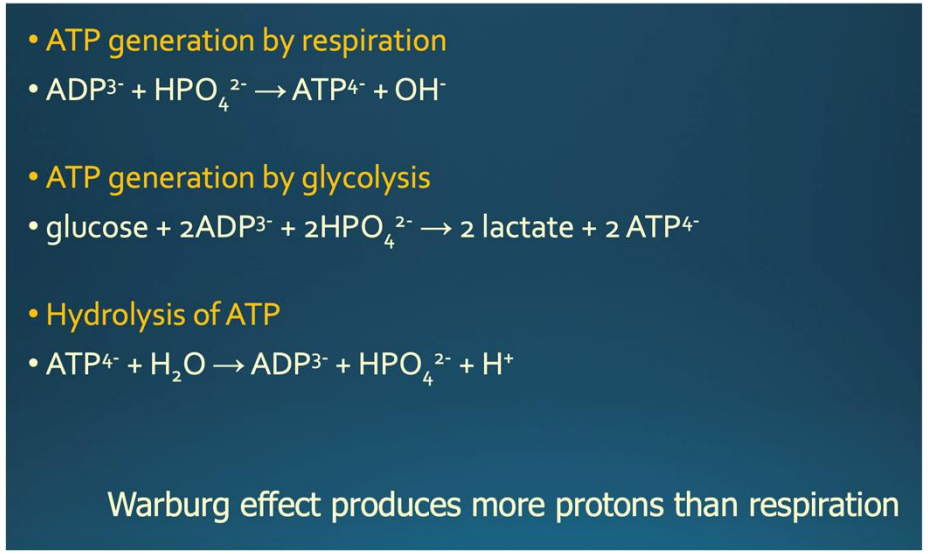
降解一个甘油三酯
将产生三个H^+



瓦博格效应

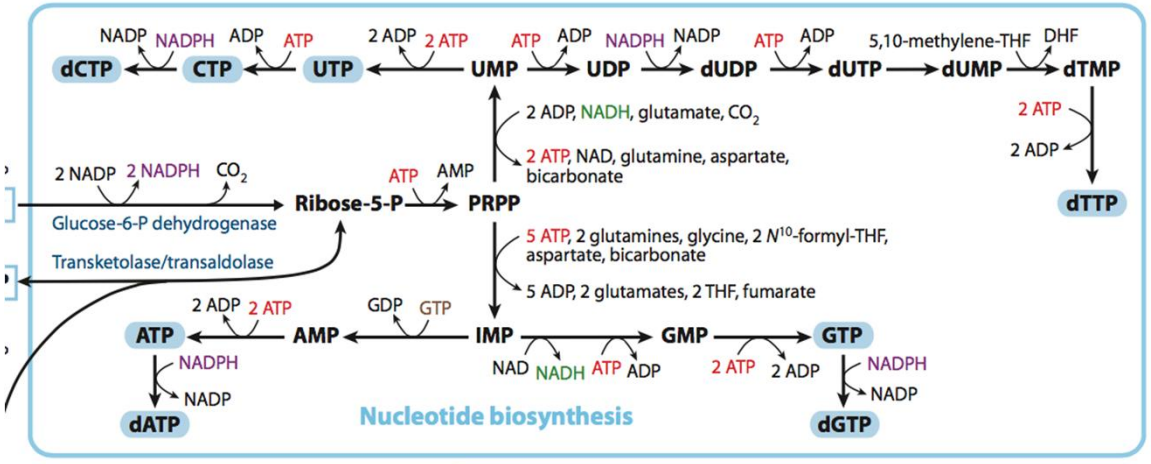
- 相比于正常细胞使用有氧呼吸，肿瘤细胞更喜欢用发酵的方式产生ATP，尽管前者是后者效率的18倍 （Warburg，1927）





核苷酸合成的代谢重编程

- 相对于正常细胞从血液中摄取核苷酸，来合成DNA，每种肿瘤都主要依赖从头合成来产生核苷酸 （1970s）
- 肿瘤合成的嘌呤个数一般（远）高于嘧啶个数



嘌呤

嘧啶

Sun, Zhou, et al. Cancer Research, 2020.

Pyrimidine *de novo* synthesis:

5 ATP + 2 H_2O + 2 glutamine + aspartate + 5-phospho-D-ribose-diphosphate -> dCDP + H_2CO_3 + 5 H^+;

5 ATP + H_2O + glutamine + aspartate + 5-phospho-D-ribose-diphosphate -> dTDP + H_2CO_3 + 3 H^+.

Pyrimidine salvage pathway:

2 ATP + H_2O + 2-deoxycytidine -> dTDP;

2 ATP + 2-deoxycytidine -> dCDP + H^+;

2 ATP + thymidine -> dTDP + H^+.

越恶性的肿瘤，从头合成的比例越高

Purine *de novo* synthesis:

5 ATP + GTP + H_2CO_3 + glycine + 2 aspartate + 5-phospho-D-ribose-diphosphate -> dADP + 8 H^+;

5 ATP + NAD^+ + H_2CO_3 + H_2O + glycine + aspartate + glutamine + 5-phospho-D-ribose-diphosphate -> dGDP + NADH + 9 H^+.

Purine salvage pathway I:

ATP + 2-deoxyadenosine -> dAMP + H^+;

ATP + 2-deoxyguanosine -> dGMP + H^+;

Purine salvage pathway II:

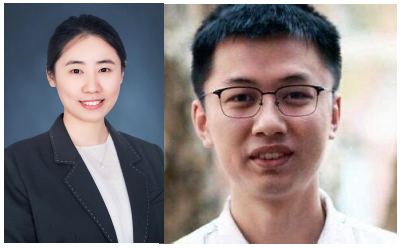
2 ATP + adenosine -> dADP + H_2O + H^+;

ATP + GTP + adenosine -> dADP + H_2O + H^+;

2 ATP + NAD^+ + 2 H_2O + guanosine -> dGDP + NADH + 2 H^+;

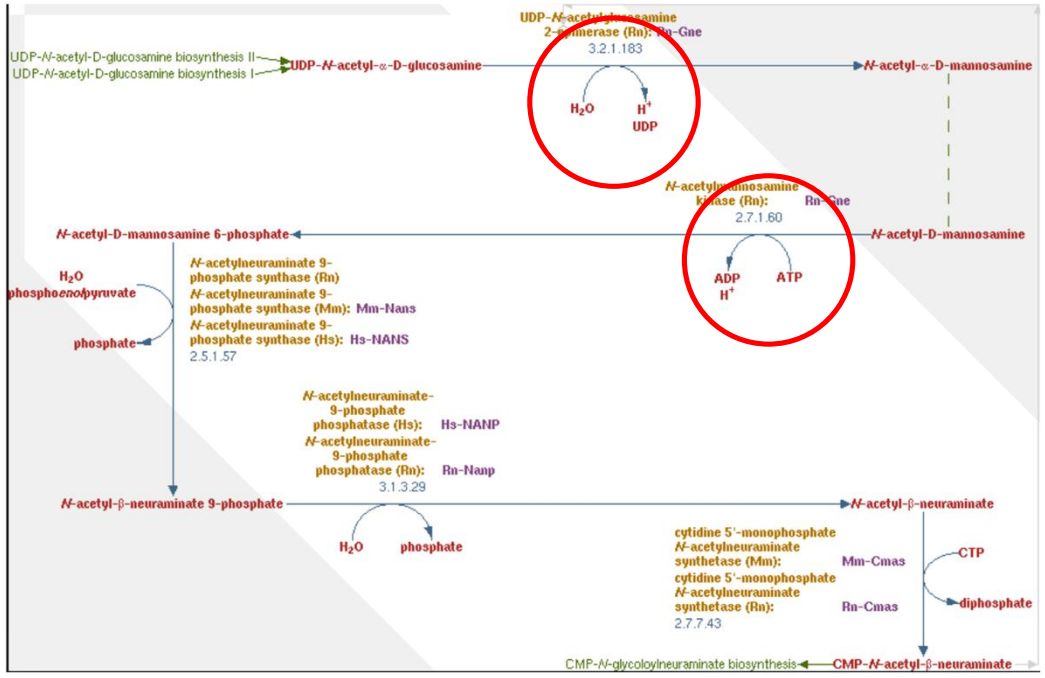
ATP + guanosine -> dGDP + H_2O.

孙慧妍　　周易

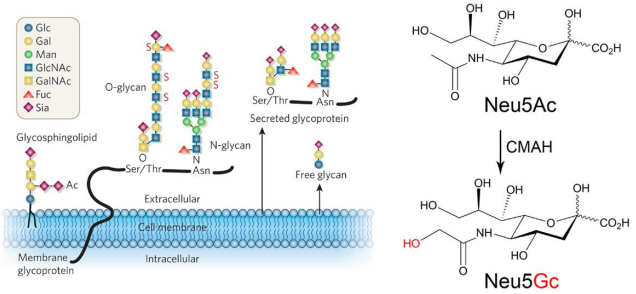


Increased Sialic Acid Production

- All cancers tend to gradually increase their sialic acid and ganglioside synthesis as the disease advances

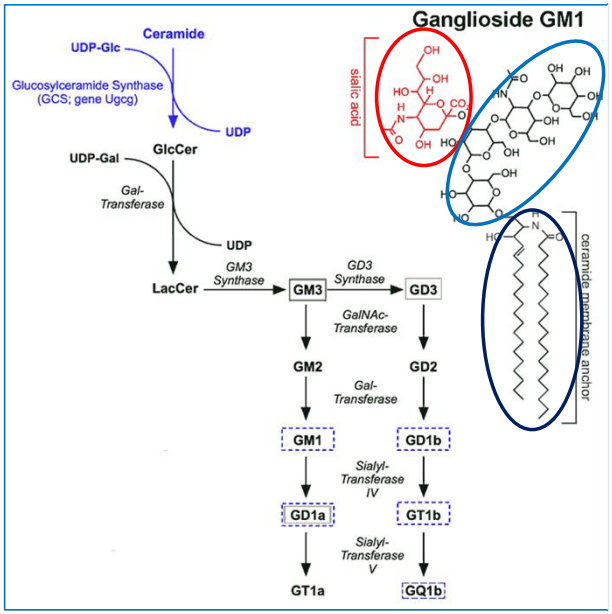


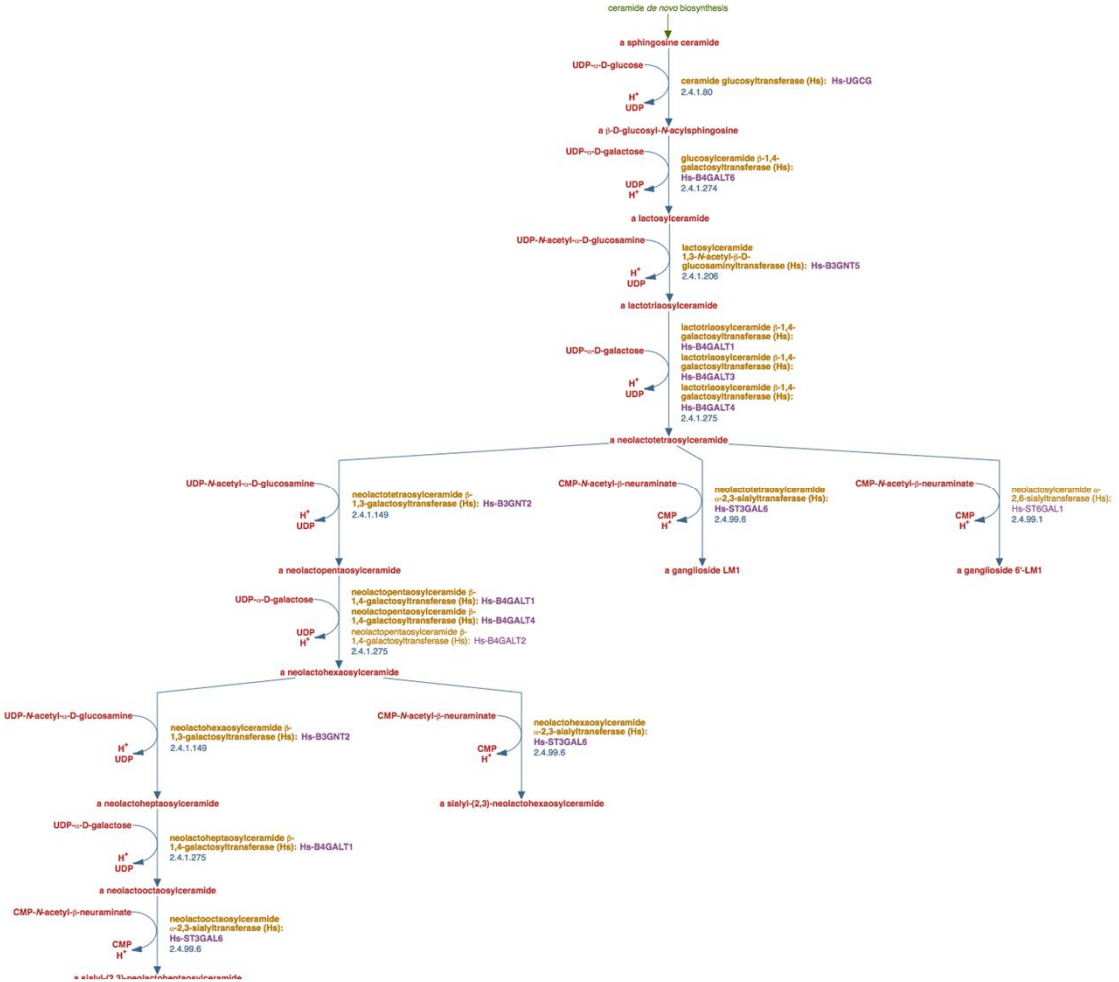
Sialic acid synthesis



Two net protons are produced when a sialic acid is biosynthesized

Increased Ganglioside Synthesis





The synthesis of gangliosides produces numerous protons

Increased Phosphorylation in Cancer

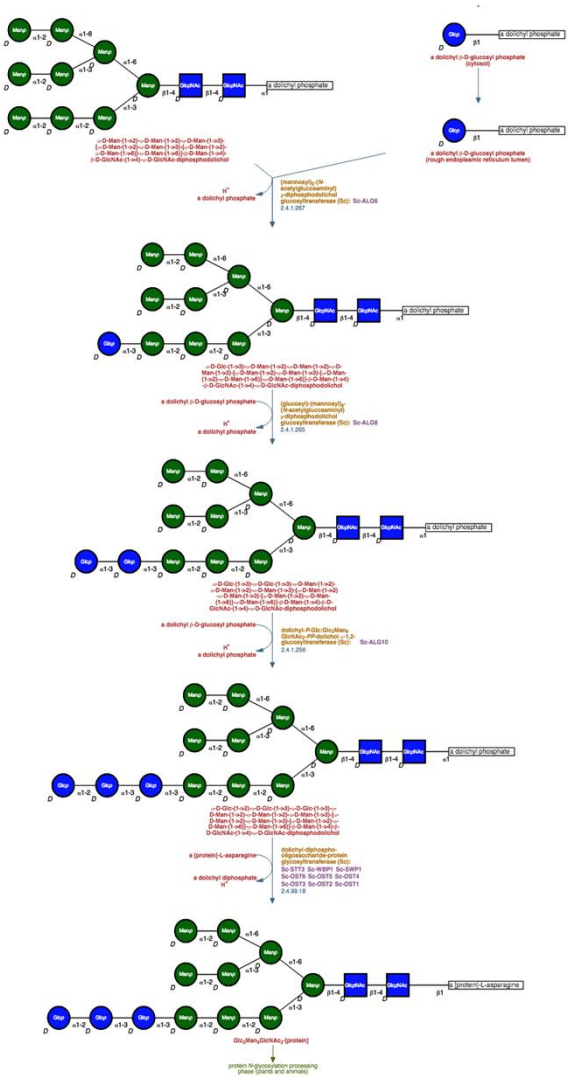
- Cancer cells tend to considerably increase the activities of kinases, giving rise to increase in phosphorylation

- The process of phosphorylation generally produces one proton for each act as it requires the following ATP hydrolysis reaction

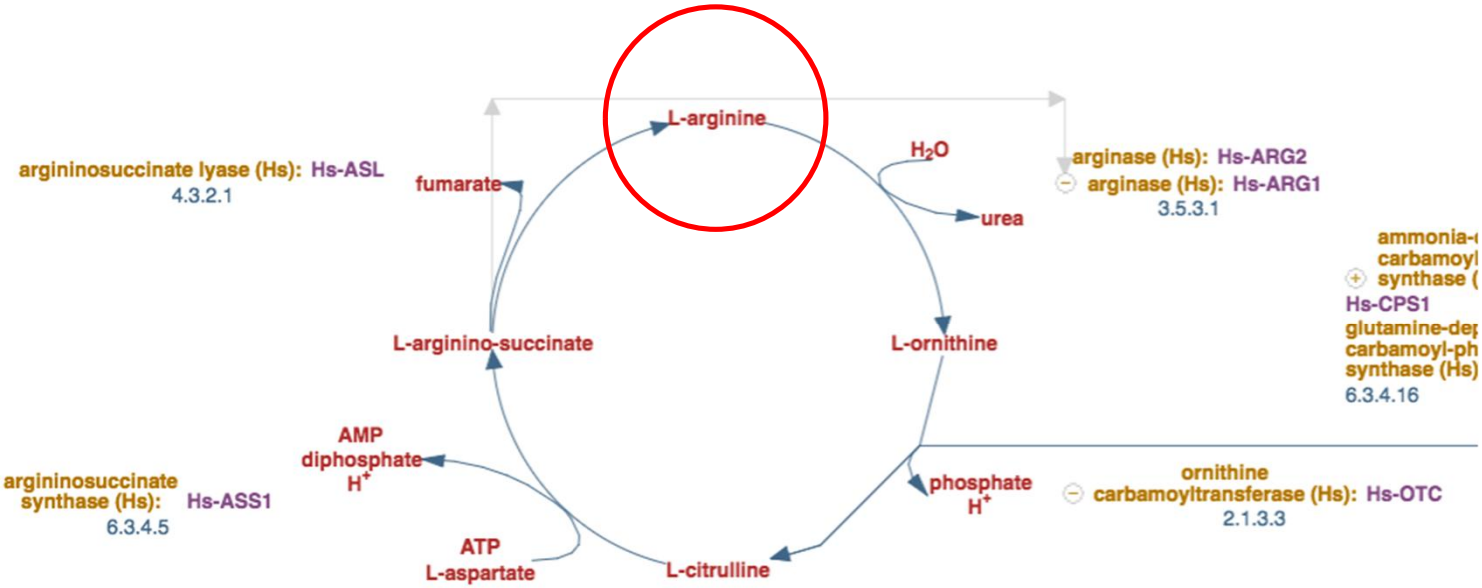
- $ATP \rightarrow ADP + H^+$

Increased Glycosylation in Cancer

- All cancers have increased glycosylation
- Numerous protons are produced per glycosylation



Repressed/Inhibited Urea Cycle



Urea cycle requires keeping a large pool of arginine, hence repressed or inhibited

The Most Mutated Amino Acids

mutations around hotspot site in five typical genes and (v) distribution of silent and missense substitutions. We observed that R → H substitution is dominant in drivers followed by R → Q and R → C whereas E → K has the highest preference in passenger mutations. A set of 17 mutations including R → Y, W → A and

Arginine: #1

Lysine: a distant #2.

Two most basic amino acids

Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease

Volume 1862, Issue 2, February 2016, Pages 155-165

Exploring preferred amino acid mutations in cancer genes: Applications to identify potential drug targets

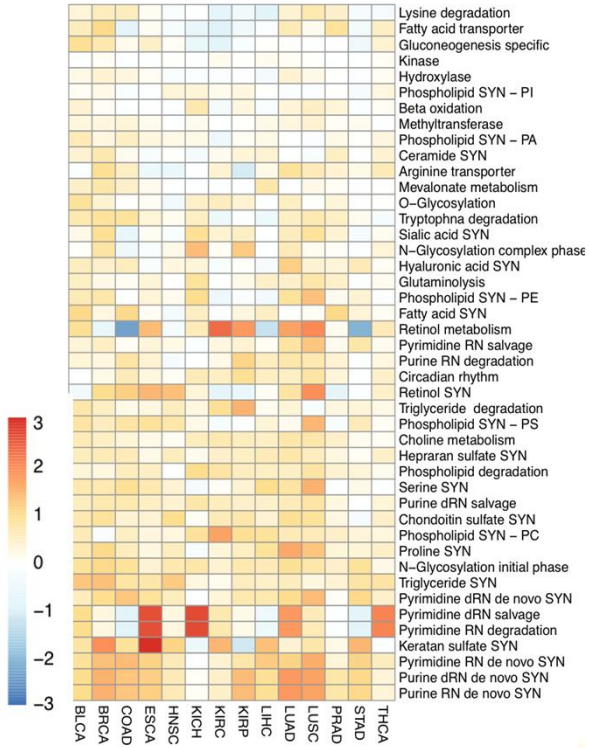
P. Anoosha, R. Sakthivel, M. Michael Gromiha

Extensive Metabolic Reprogramming in Cancer

- We have analyzed ~50 reprogrammed metabolisms across 14 cancer types, 7,000+ samples in TCGA

- Found that every reprogrammed metabolism examined produces more protons than the original metabolism.

- And yet cancer intracellular pH goes up, according to the literature!

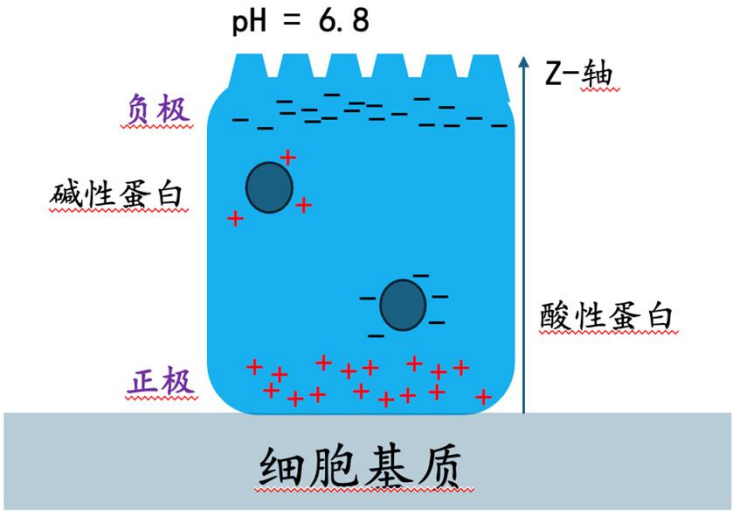


一个极重要但长期被忽视的因素：pH

- pH为细胞的化学系统提供了一个舞台。当pH改变，细胞内的化学系统将发生深刻的变化，包括死亡
- 例如：pH的改变将改变蛋白的折叠、功能，酶促反应的动力学及热力学性质；甚至改变组蛋白在染色体中packing密度
- **特别重要的是**：胞外的酸性化将改变钠、钾离子在细胞内外的平衡，进而改变细胞膜的**电势**，及大量进出细胞物质的**平衡**，逐渐使局部问题演化为全身问题

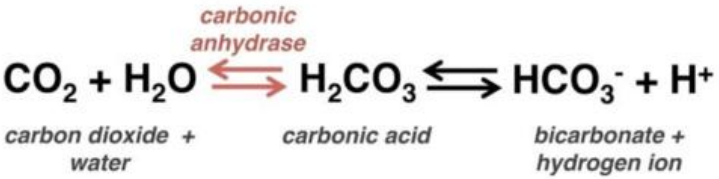
pH在细胞生物学中的重要性

- 人体细胞（如上皮细胞），有一个**电场**，一端为负电，一端为正电
- 这一电场与pH共同给细胞浆内带电分子（如蛋白）沿Z-轴定位
- pH的改变将影响蛋白沿Z-轴的定位，使原本定位在附近的蛋白（同一个通路的蛋白），沿Z-轴分开



pH的稳定

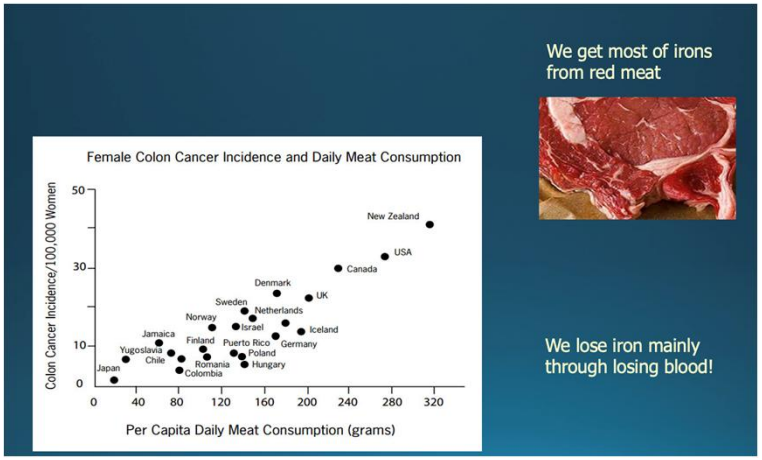
- 由于pH的重要性，细胞使用两套系统来维持细胞pH的稳定
  - **基于碳酸氢根的pH缓冲区**



  - **H^+泵或等价分子**
- 两者都仅有很有限的缓冲能力，即都不能在持续碱性或酸性化的条件下，维持细胞pH的稳定
  - pH缓冲区的容量有限
  - 而持续使用H^+泵会破坏细胞的电中性或其它离子的稳态

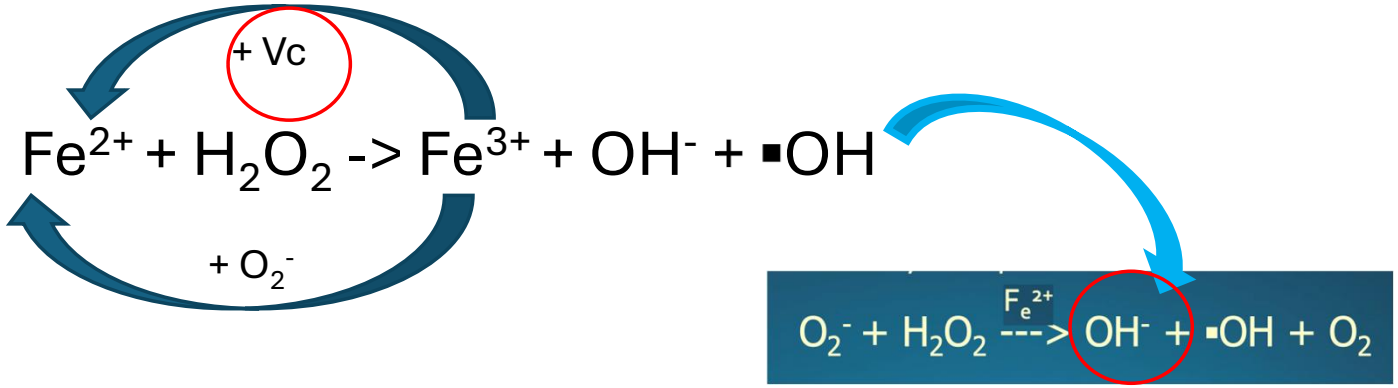
寻找碱性化的来源

- 慢性炎症被广泛接受为与肿瘤有因果关系，但具体关系如何并不清楚
- 从化学的角度，慢性发炎 == 免疫细胞大量释放(H_2O_2，O_2^-)
- 不同人群的肿瘤发病率
  - 男人 vs. 女人 3:2
  - 食肉者 vs. 素食者 3:1
  - 健康人 vs. 地中海贫血症 3:1
- 这些都表明：铁在肿瘤形成中起到作用
  - 铁主要来自红肉
  - 铁进入我们体内后，将不会离开（除非失血）



肿瘤细胞中的芬顿反应

- 当慢性发炎（免疫细胞大量释放H_2O_2，$\cdot O_2$） 遇到Fe^{2+}局部积累，将发生**芬顿反应**



- 这将导致细胞内持续被碱性化
- 吃维生素C似乎会加速癌症的发展

孙慧妍　张驰

Sun, Zhang, et al, JMCB, 2018; Sun, Zhou, et al. Cancer Research, 2020.

芬顿反应及相关代谢重编程

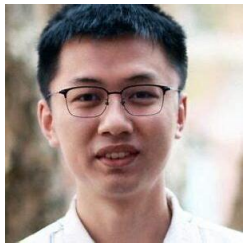
- 我们预测了：TCGA中的每个肿瘤组织都在其细胞浆存在芬顿反应
- 进而预测了每个肿瘤中的酸性化代谢重编程是用于维持受芬顿反应影响的细胞浆pH的稳定

$$R\ (OH^-,\ FR) \approx \sum_i R\ (H^+,\ Mi)$$

- 在所有肿瘤中，最主要的酸性化代谢重编程为核苷酸从头合成及唾液酸合成

$$R\,(OH^-, FR) \approx R\,(H^+, NT) + R\,(H^+, SA) + \varepsilon$$

周易



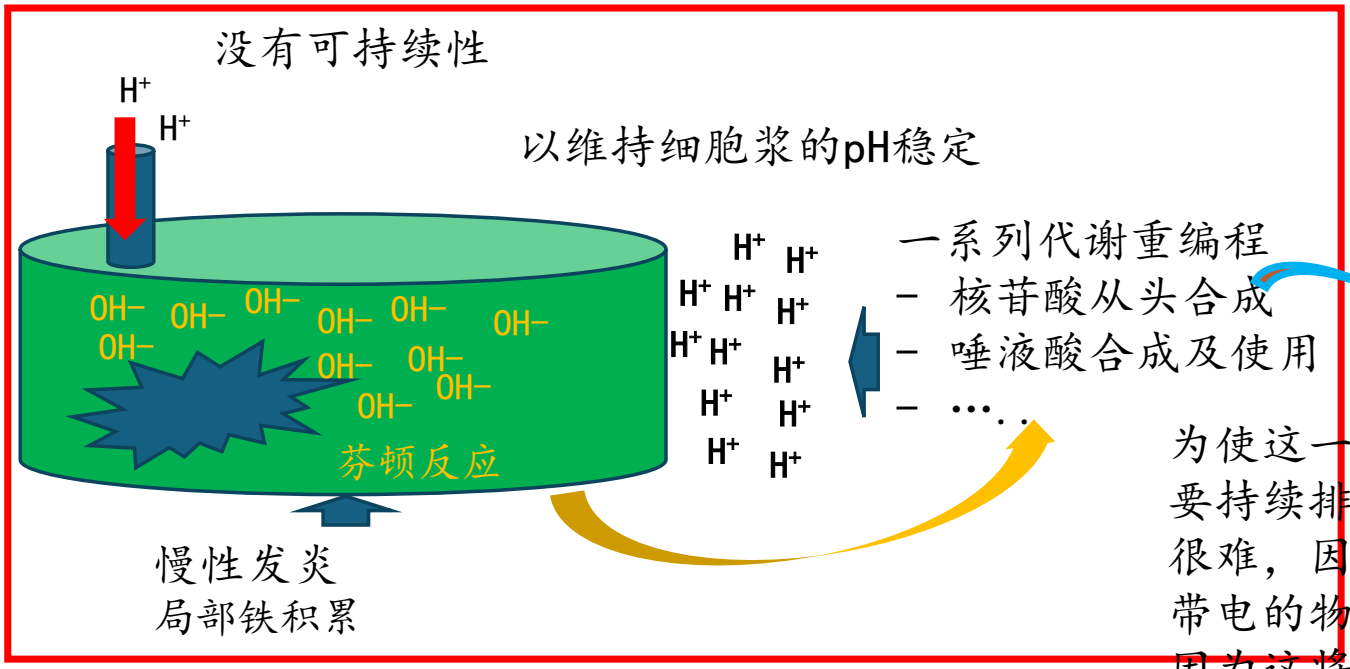
Zhou, et al. GPB, 2022.

Cancer types	Significance of up regulated proteasome genes	Significance of up regulated mRNA degradation genes	Averaged R^2 value	Significance of the M-M model
BLCA	4.94E-07	5.96E-06	0.697	0.000117
BRCA	1.12E-07	0.00828	0.756	0.000505
COAD	4.86E-07	8.46E-14	0.783	3.75E-05
ESCA	1.02E-19	2.92E-17	0.709	0.00144
HNSC	3.00E-04	4.92E-06	0.702	0.00145
KICH	2.20E-07	0.0376	0.814	0.00844
KIRC	0.0794	0.741	0.852	7.50E-05
KIRP	3.28E-05	0.292	0.842	2.01E-06
LIHC	0.0056	7.83E-05	0.761	0.000702
LUAD	8.50E-10	7.74E-07	0.772	0.000431
LUSC	2.42E-09	9.71E-12	0.701	0.0018
PRAD	6.02E-05	0.000717	0.828	8.69E-06
STAD	2.28E-11	1.24E-09	0.774	0.000877
THCA	8.41E-05	1	0.864	5.01E-05

孙慧妍　张驰

初始模型

一个大胆的假设：细胞的持续增值是为了将为救命而持续合成的核苷酸快速移出，以保证细胞可以继续不断合成核苷酸 – 细胞 ← DNA^- + 组蛋白$^+$

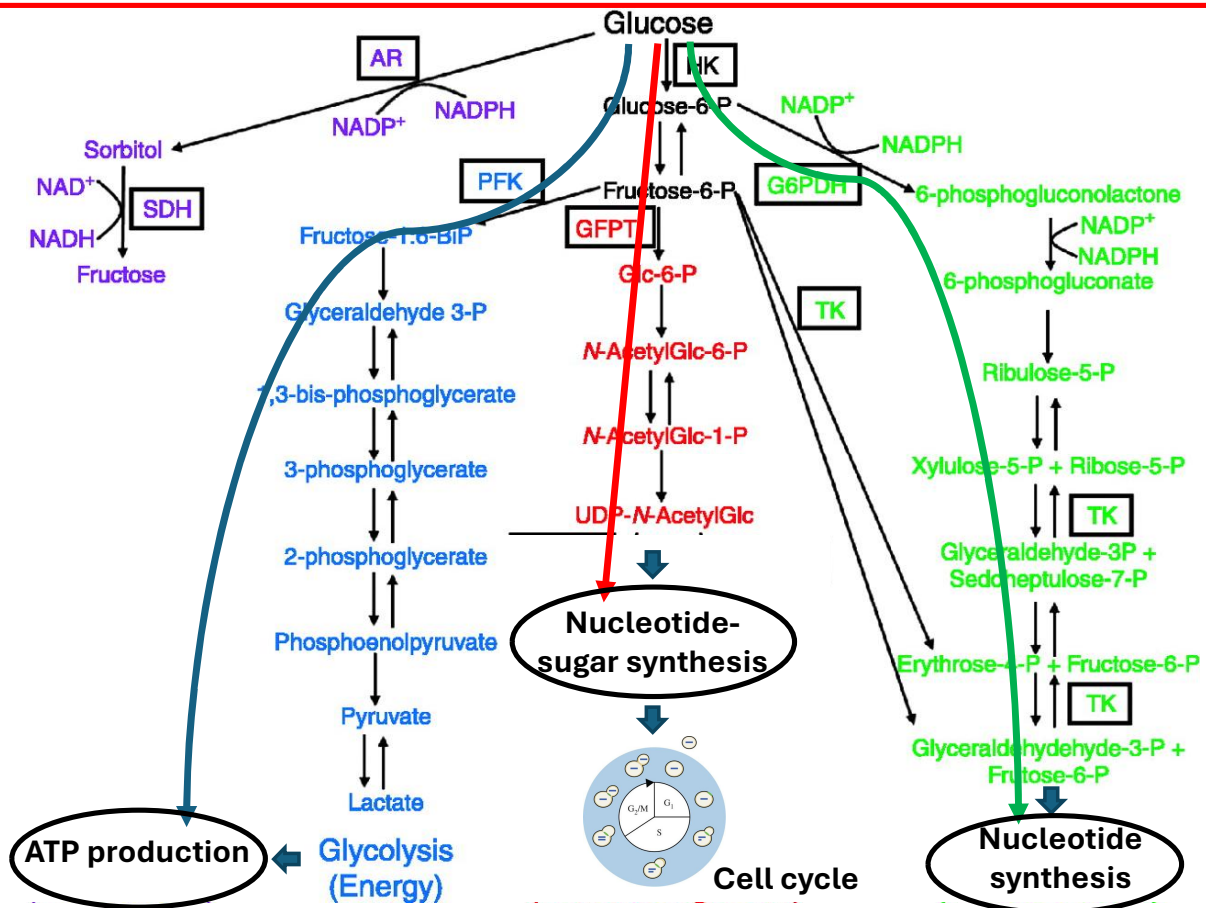


为使这一过程可持续，细胞需要持续排除合成的核苷酸但这很难，因为核苷酸带负电，而带电的物质不能持续进/出细胞，因为这将破坏**细胞的电中性**

.

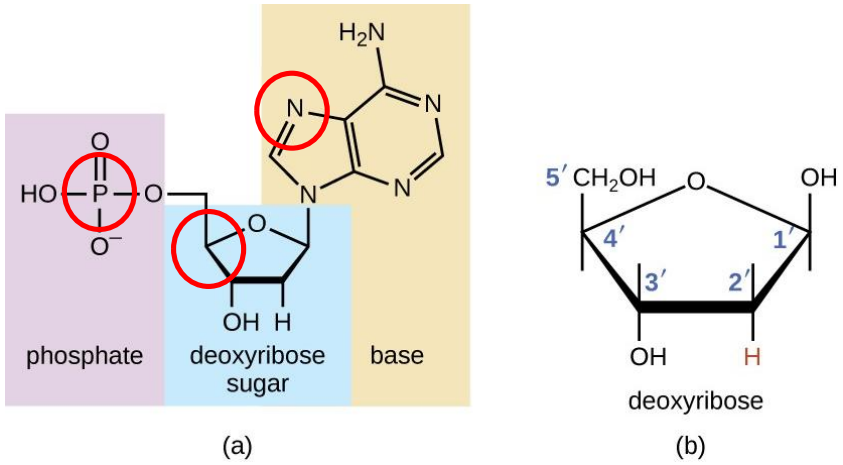
细菌的增殖

只要有食物，细菌会不断吃，首先产生ATP。当其浓度高到一定程度后，细胞转而合成核苷酸；其浓度高到一定程度，细胞开启细胞周期，并合成DNA、蛋白、脂、糖，形成一个细胞，即核苷酸浓度推动细胞周期



Cell Division of Unicellular Organisms

- It is considered that the concentration of UDP-sugar is the cue for cell cycle initiation in unicellular organisms like yeast
- The possible reason that UDP-sugar is used is that it consists of carbon, nitrogen and phosphate, i.e., when all the essential elements have adequately high concentrations for cell division



Hypothesis

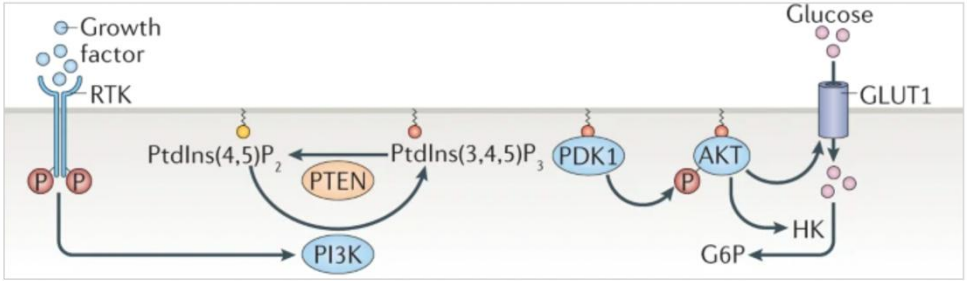
- Is it possible that cancer cells utilize a program similar to the cell division program driven by concentration of nucleotide-sugar, as way to remove rapidly synthesized nucleotides?

- One issue is that human cell division is not driven by accumulation of nucleotides or variants

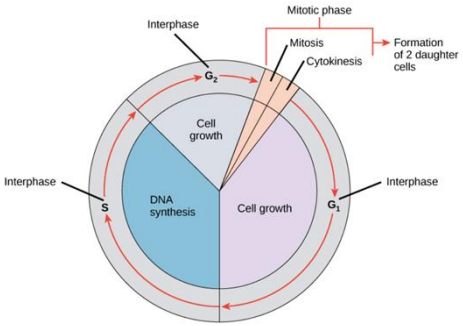
- Question: what changes do human cells need to have, to make such cell division possible?

Cell Division in Human

- Cell division is a top-down process, which is initiated by growth signals and coordinated among cell cycle progression, nutrient balance, cell size, and redox homeostasis



- PI3K/PTEN are the key regulator of cell

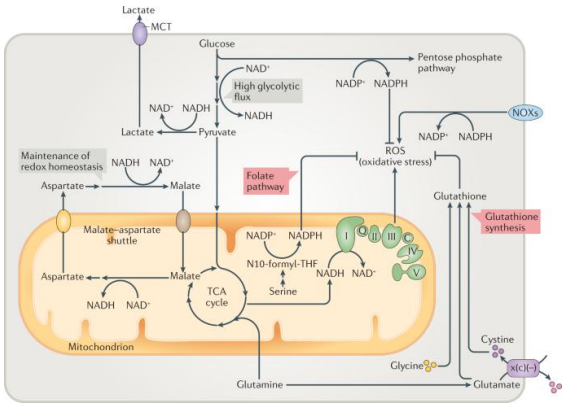


Nutrients

- Glucose
- Amino acids
- Lipids
- Nucleotides

Cell size

Redox homeostasis



Cell Polarity

- Cell polarity provides the supporting infrastructure to functions related to localization, transportation, organization of molecular machinery
  - E.g., protein localization, transport (import, export, secretion, endocytosis, exocytosis), placement of organelles
  - Cytoskeletal structures
  - Lysosome and Golgi are centers of endocytosis and exocytosis, respectively

- Cell polarity is dynamic; in different phases of a cell, different functions need to be performed, hence a different type of cell polarity is built
  - Static state, division, differentiation, migration ….
- Conversely, different cell polarities support a different set of functions

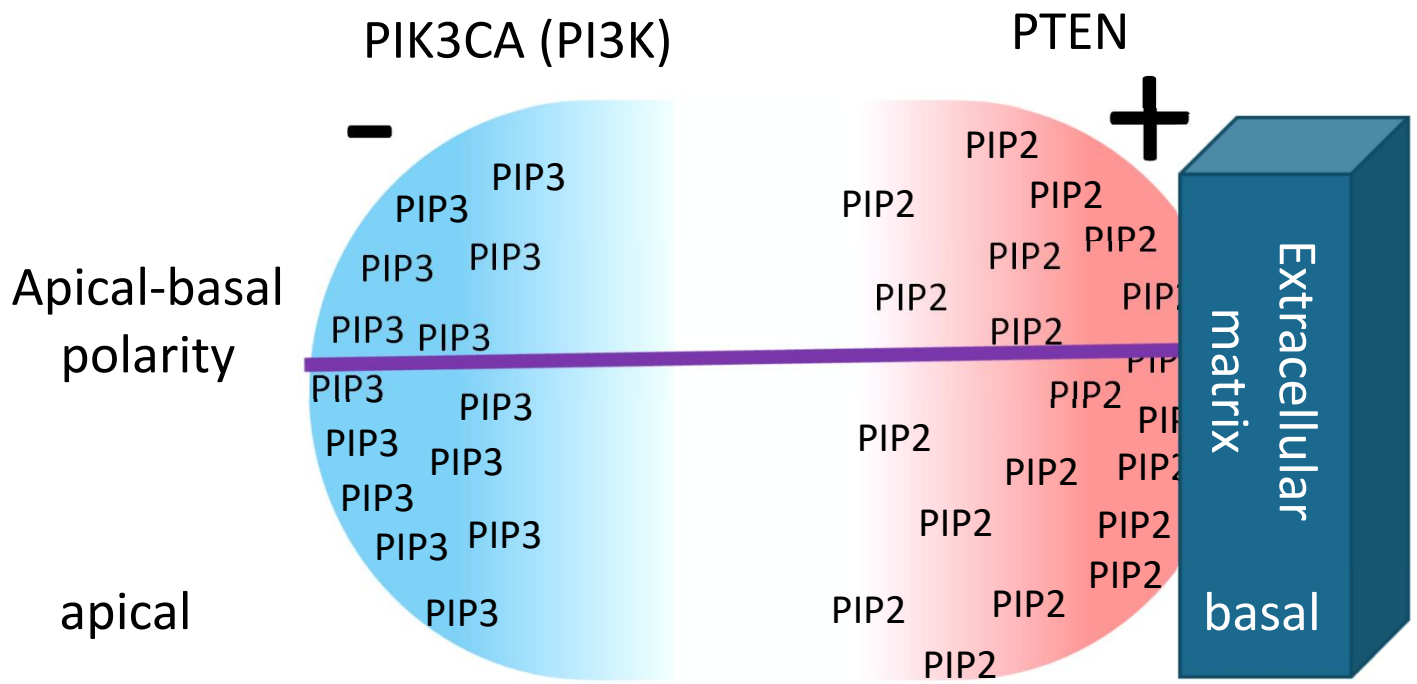
Cell Polarity

- Cells that do complex functions require a sophisticated cell polarity
  - Like freeway, subway, high-speed train system, overpass in a big city like Shenzhen while a village needs only a few roads to support its simple functions

- Cell polarity defines a cell type!

Human Cell Polarity

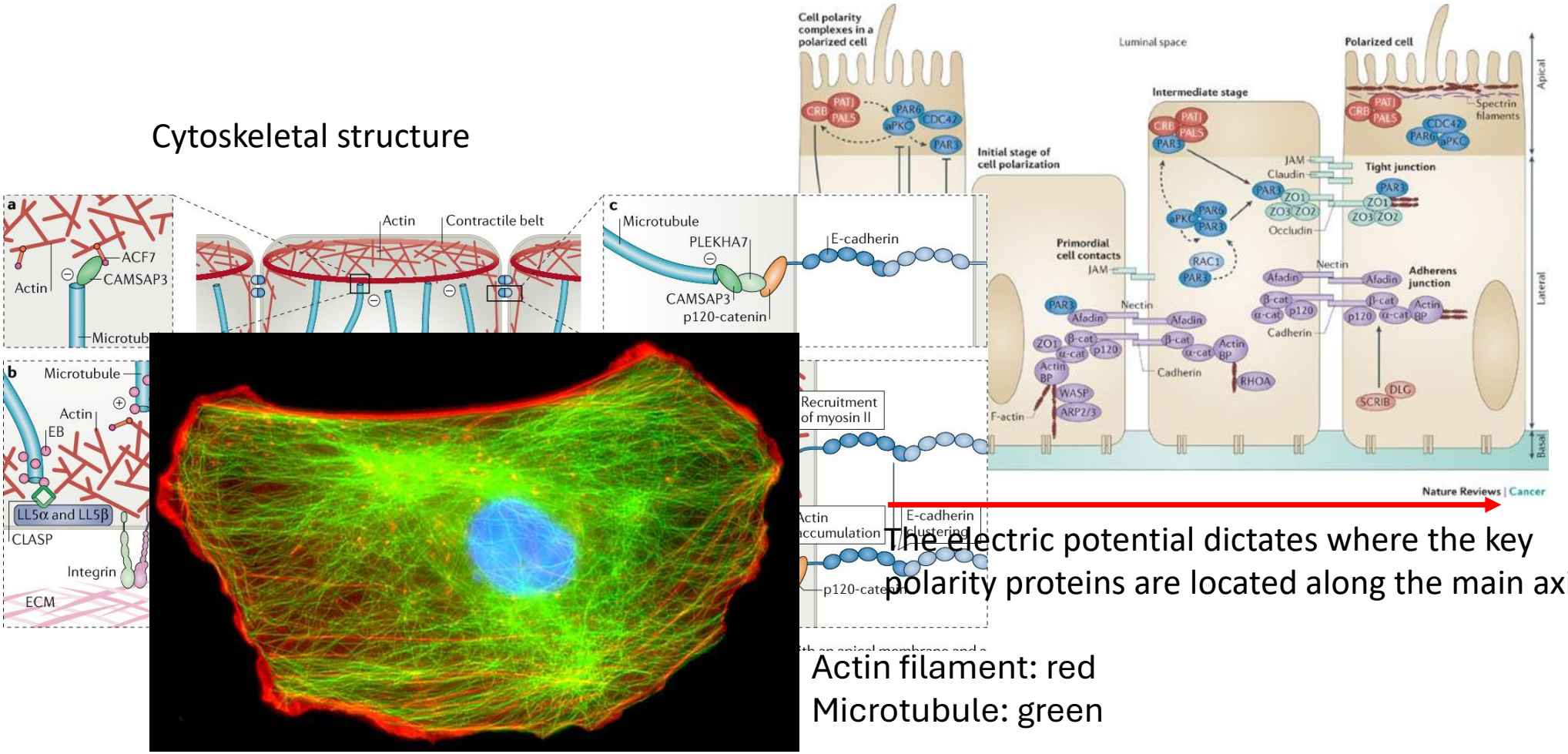
- In human, the foundation of cell polarity is differential electric charges at the two poles



Apical-Basal Polarity

The actin filaments and microtubules serve as the infrastructure for localization, transportation and cell shape (along with intermediate filaments), which are regulated by PIPs

Cytoskeletal structure



The electric potential dictates where the key polarity proteins are located along the main axis

Actin filament: red
Microtubule: green

Mutations in Cancer Genome

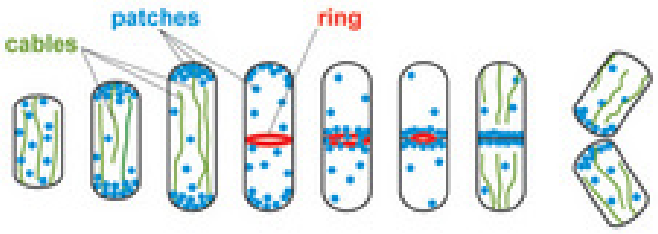
- We have conducted a systematic analysis of all the mutated genes across all genomes of a cancer type in TCGA

- We found that 30-40% of the mutated genes are part of the polarity system in support of cell division of human cells

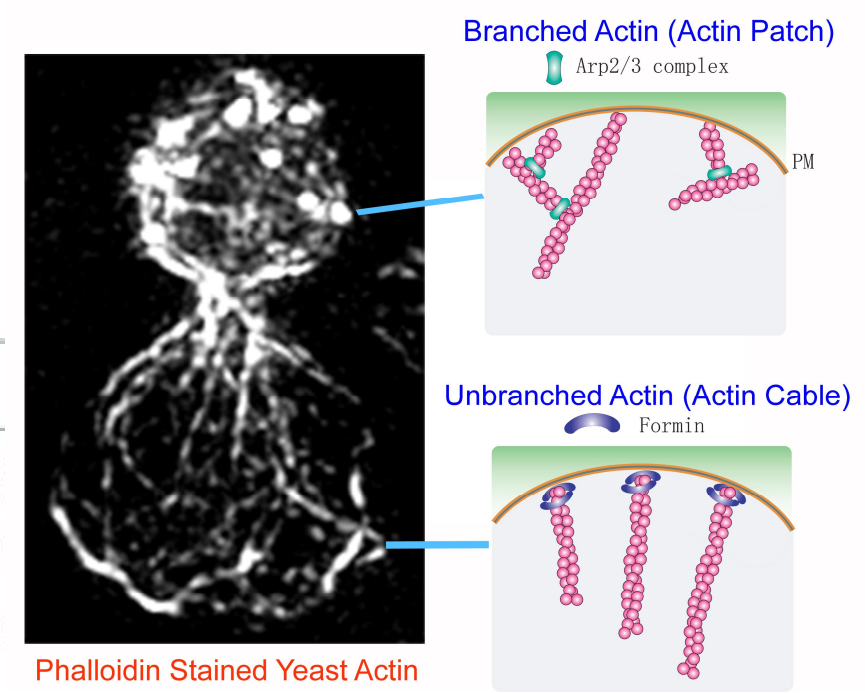
Cell Polarity in Yeast

- Yeast has a more complex cell polarity system compared to bacteria

- Actin-like proteins provide the basis for polarity structure for yeast cells



Actin structure	*Nucleation factor*	*Actin organization*	*Myosin motor*
cables:	For3p (formin)	long unbranched bundles	Myo52p (type-V)
rings:	Cdc12p (formin)	short unbranched bundles	Myo2p, Myp2p (-II) Myo51p (-V)
patches:	Arp2/3 complex	branched network	Myo1p (-I)



Cancer's Polarity System

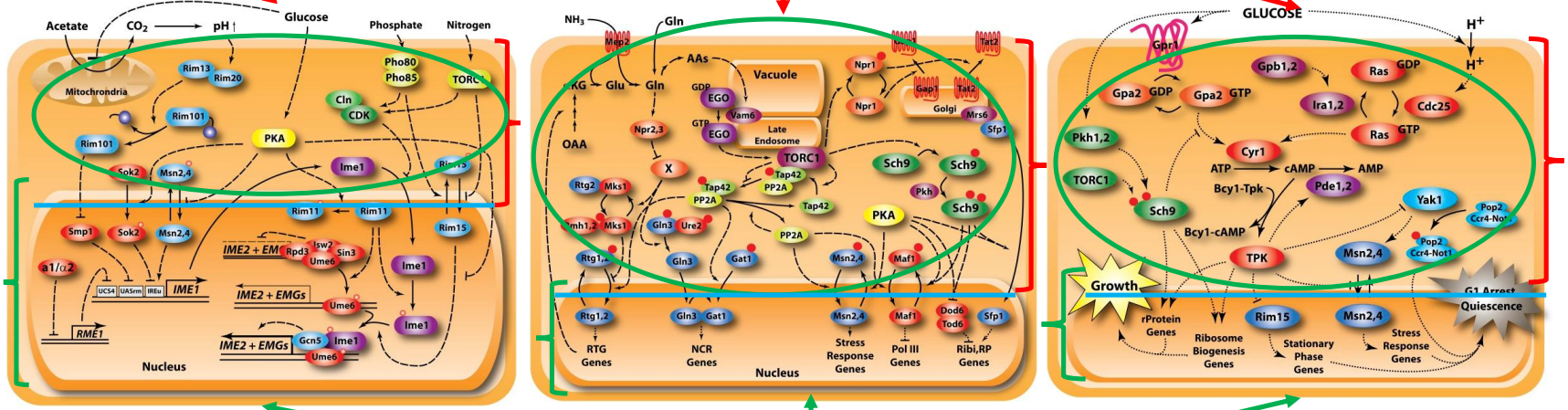
- Mutations to simplify cell polarity, hence cell complexity: at least 50% of all the genomic mutations in a cancer are related to simplification of human's cell polarity machinery to one like in a unicellular organism (X).
  - Many of them have been referred to as "tumor suppressor genes" such as TP53, PIK3CA, APC

- Our analyses revealed that the set of unmutated polarity genes in support of cell division is similar to that of yeast

Cell Division of Yeast

Nutrient sensing and related signaling genes

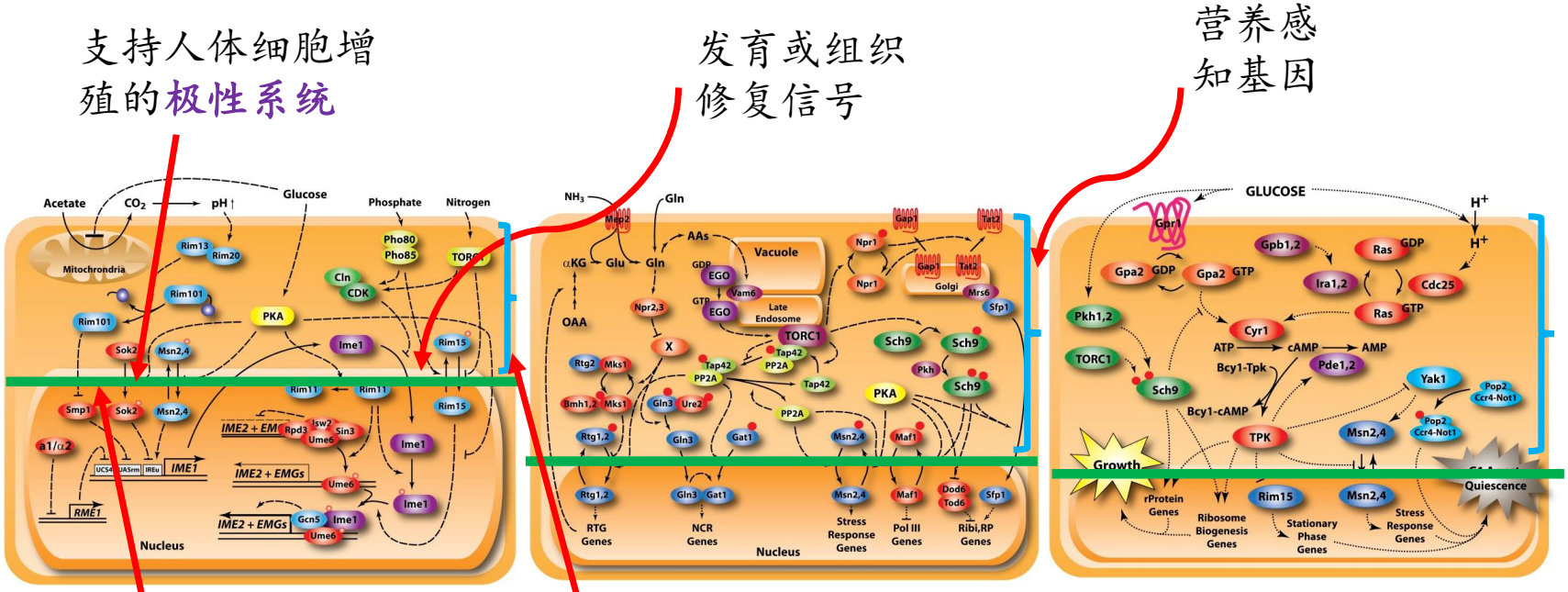
Their human homologs are all oncogenes



Cell cycle program

Yeast cell division is driven by nutrient concentrations

- 多细胞生物的增殖系统：增加了复杂的**极性系统** —— 支持细胞增殖的基础设施



抑癌基因
– 需要失活

原癌基因
– 需要过表达

肿瘤可能使用了单细胞真核生物使用的方式来驱动细胞周期，即使用各类营养浓度来驱动细胞周期？

Cancer as a “Unicellular” Organism

- Oncogenes are predominantly nutrient sensing genes in yeast as it has been reported by multiple authors
  - Majority of the proto-oncogenes are from unicellular organisms and key oncogenes such as RAS, MYC, MTOR, are related to nutrient sensing

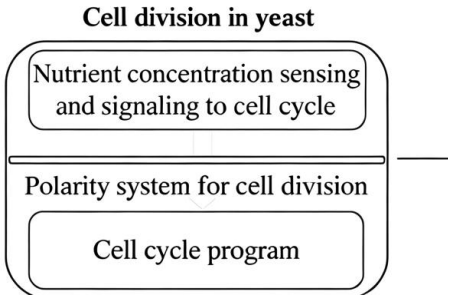
- We predict that cancer cells are highly simplified cells, like unicellular organisms to enable that nutrient concentrations can drive cell cycle for survival!

肿瘤中的基因突变

- 我们的分析发现：每个肿瘤组织中～50%的突变发生在用于定义、维护细胞极性的基因
  - 用于细胞内定位、转运、细胞间相对位置的基础设施
- 并进一步发现：肿瘤细胞的极性类似于最早的多细胞生物的极性系统
- 新的假设：染上芬顿反应的细胞为了能持续使用核苷酸从头合成来维持pH稳定，大幅简化其细胞结构，使得它能像单细胞生物一样，用核苷酸浓度来推动细胞周期及细胞增殖
  - 支持证据：所有“抑癌基因”都是极性系统的一部分，表明：简化细胞极性是使得细胞由核苷酸浓度推动细胞周期的必要条件
  - 原癌基因都来自细菌，起原始功能多与感知大分子浓度有关，如RAS，MTOR,MYC

肿瘤的细胞增殖

穆学琛　　黄振羽



不同肿瘤组织可能选取不同的突变，只要“条条大路通罗马”

细胞增殖

持续表达营养基因

大幅度基因突变

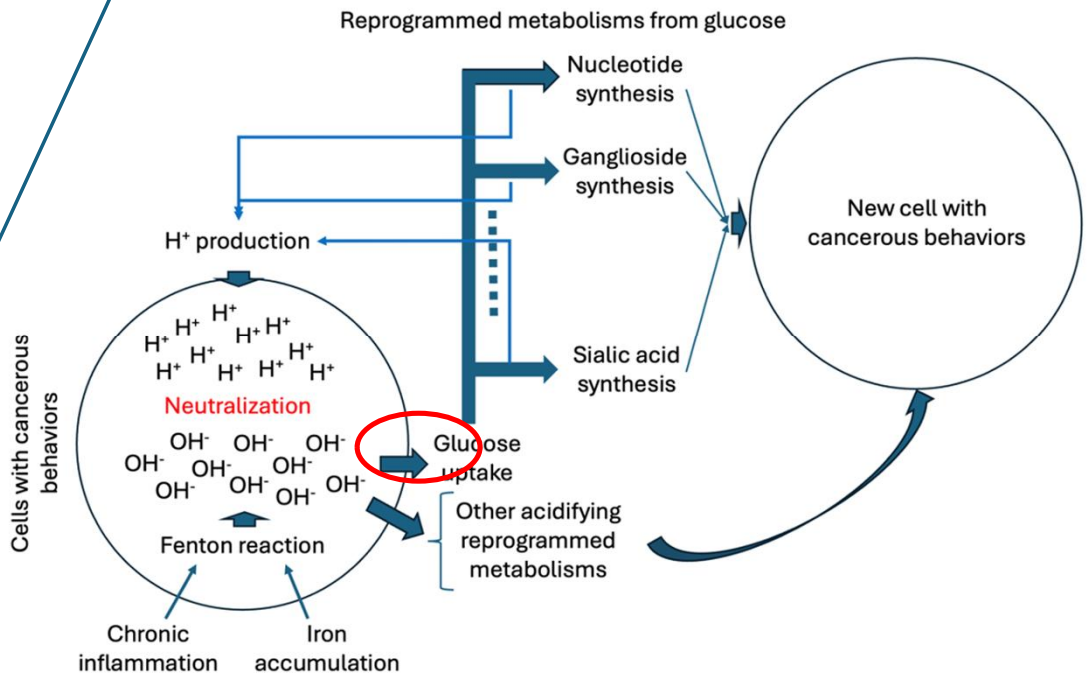
简化细胞极性系统

启动突产生变程序

酸性化代谢重编程
- 核苷酸合成& 排出
- 唾液酸合成
- 。。。

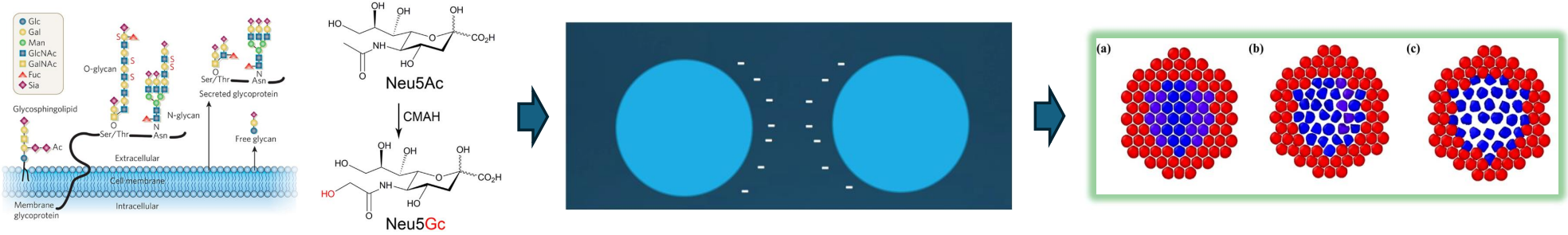
压力

细胞浆被持续碱性化

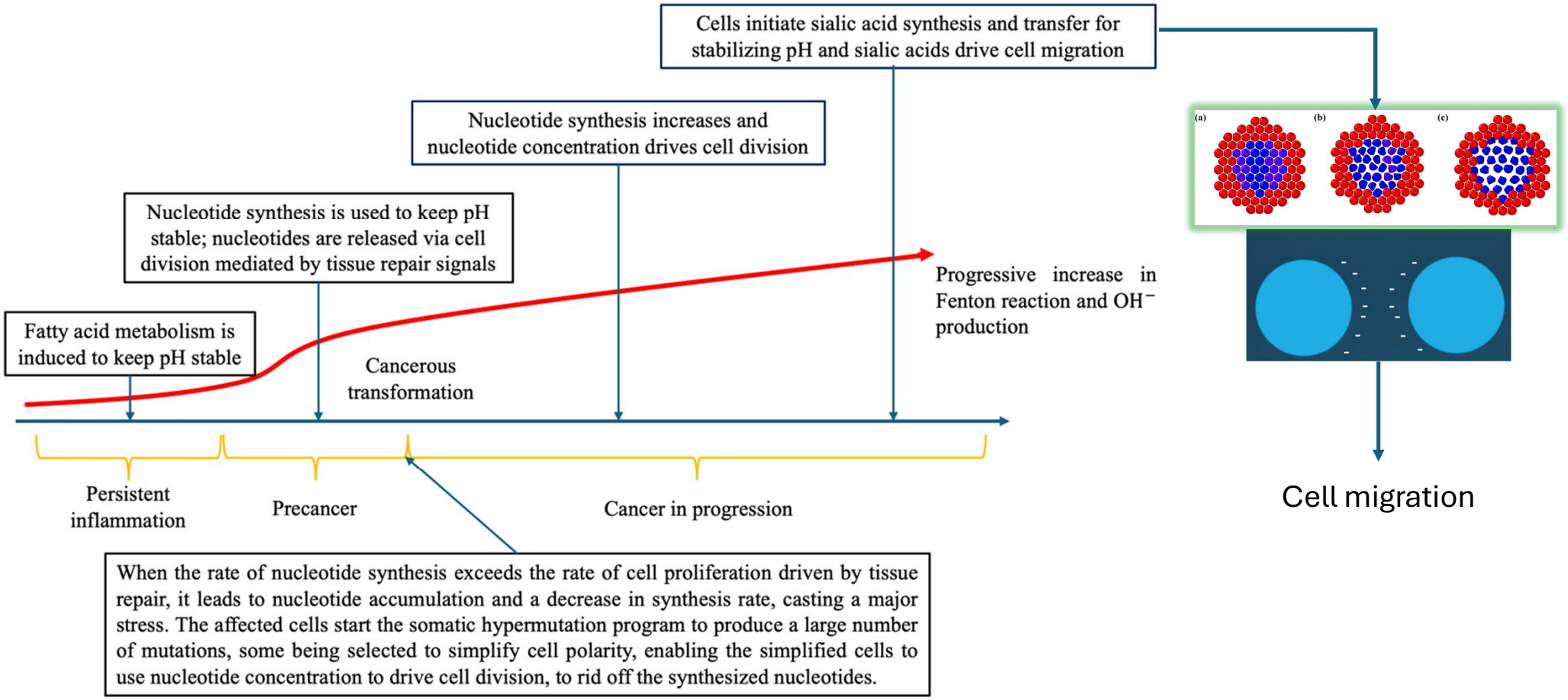


唾液酸与肿瘤迁徙

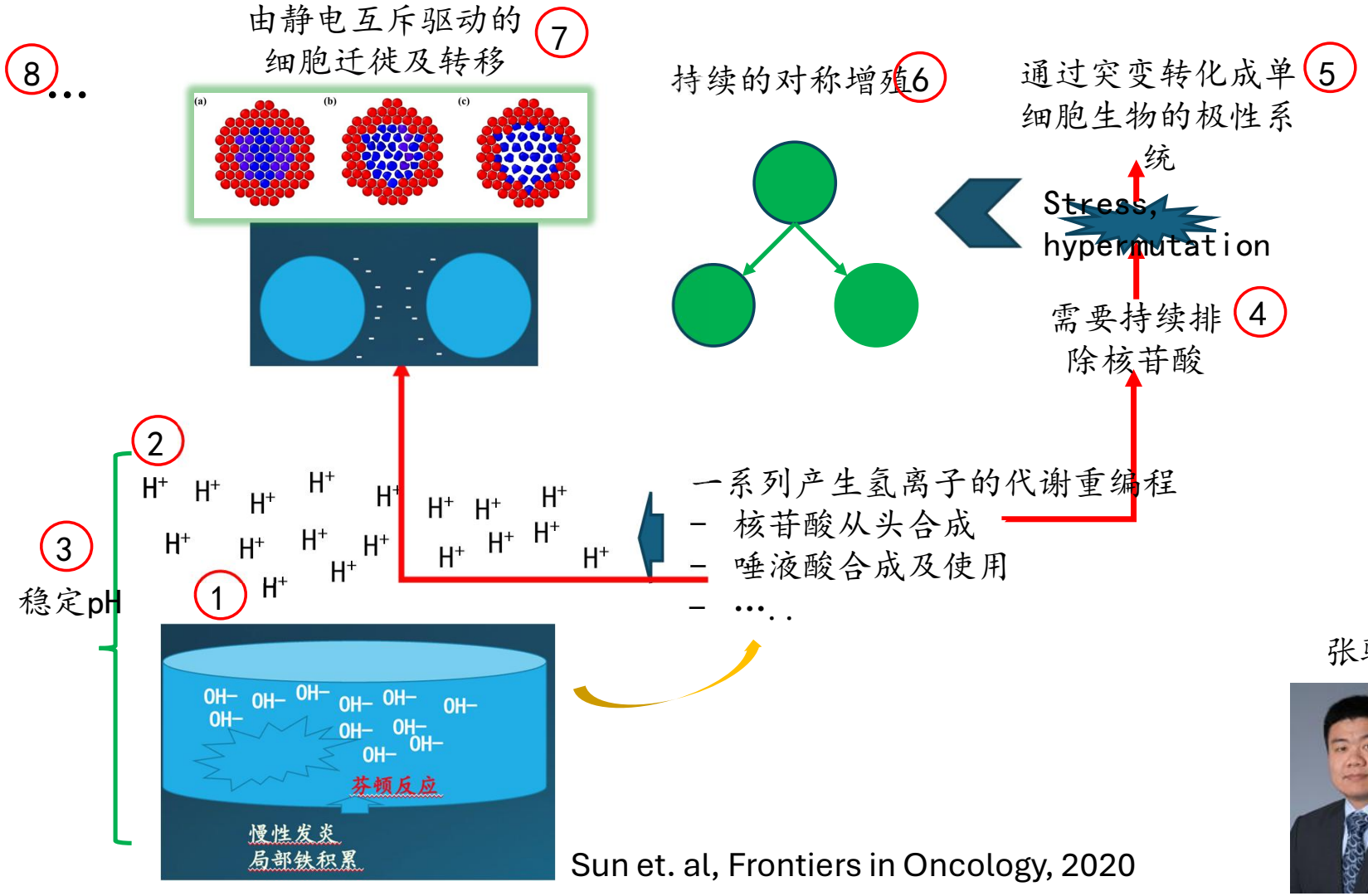
- 随着芬顿反应的持续上升，受影响的细胞启动另一个大量产生H+的唾液酸合成及转运。唾液酸带负电，被安置在细胞表面的神经节苷脂之
- 细胞表面持续积累的唾液酸导致细胞间的静电排斥。
- 逐渐导致细胞形变，最终启动上皮间质转换程序，驱动细胞迁徙



Inflammation-to-cancer Transformation: Finding Metabolic Exits for Nucleotides Synthesized for Survival



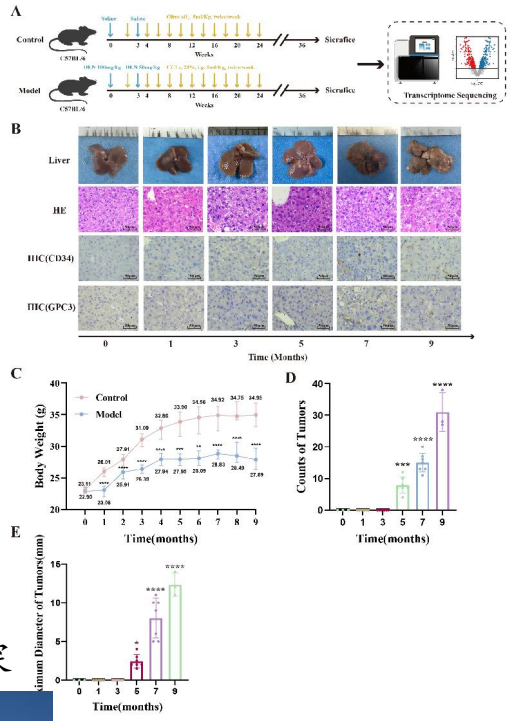
模型#1.0

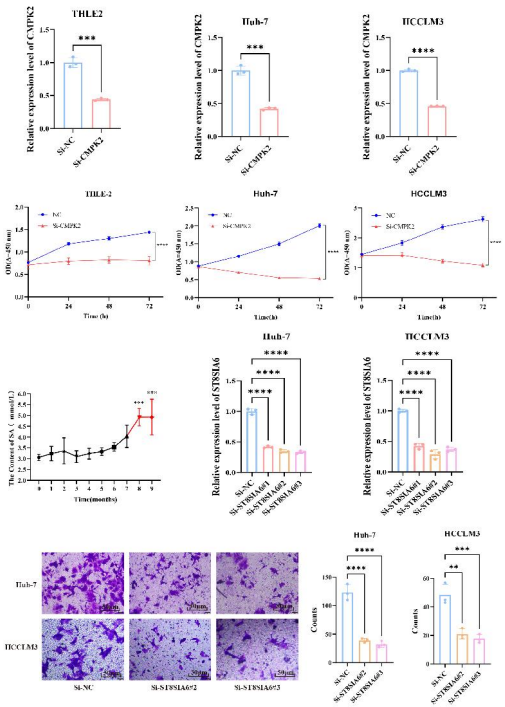


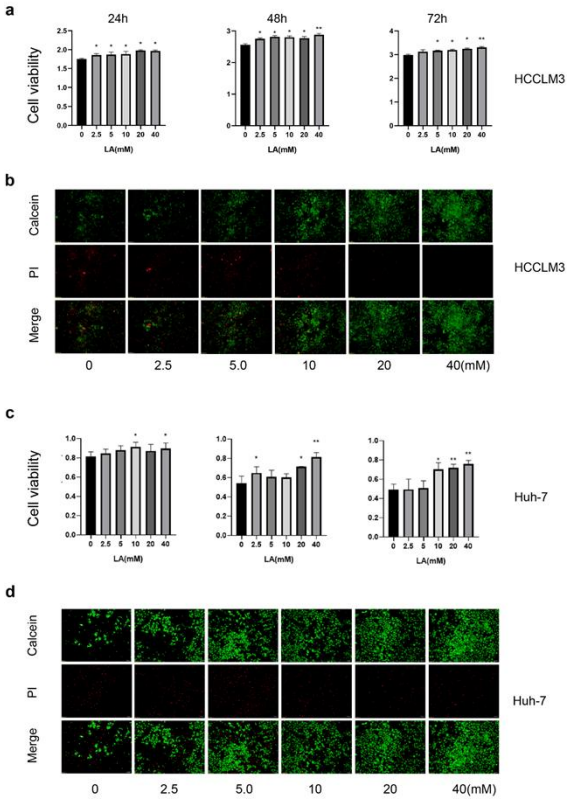
Sun et. al, Frontiers in Oncology, 2020

张驰　孙慧妍

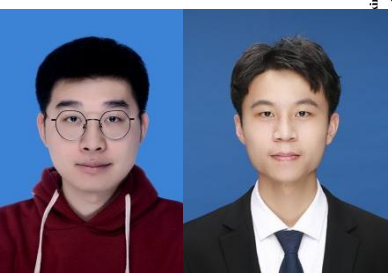
使用小鼠肝癌模型验证我们的演化模型







黄振羽　　穆学琛

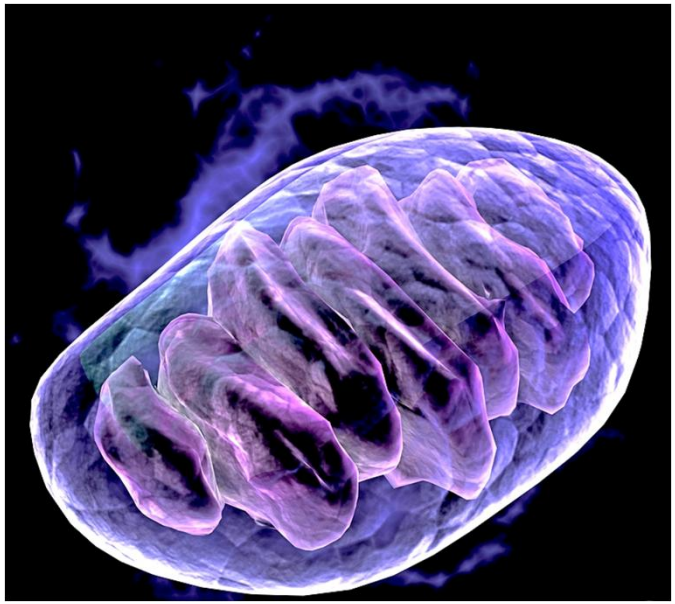


张晔

Mitochondrial Fenton Reactions

- Cancer utilizes considerably more ATPs than normal matching cells. Where do the ATPs come from?
- We predict that all cancers have Fenton reactions in mitochondria.

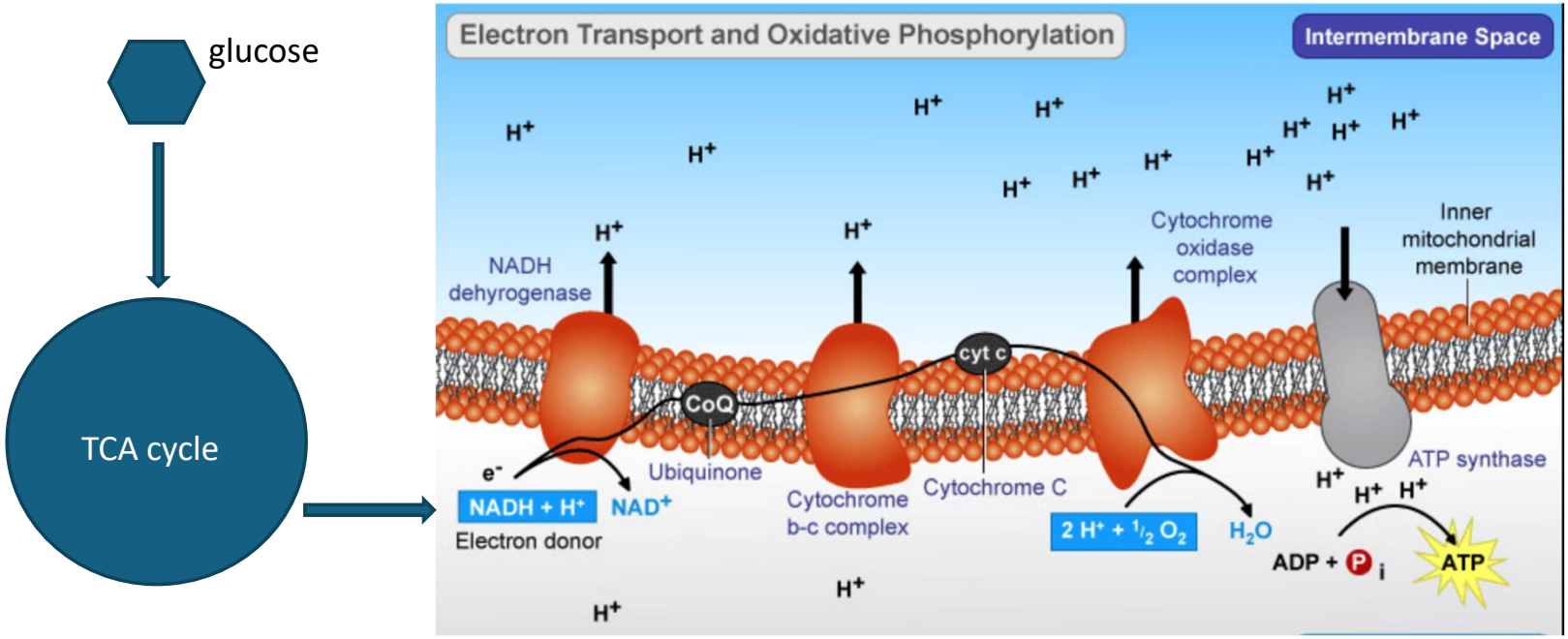
Cancer	Mitochondria		
	P-value of up regulated damage response	Averaged R^2 value	P-value of obtained R^2 value
BLCA	0.91896	0.81373	0.381
BRCA	0.00024	0.82401	0.215
COAD	<1e-5	0.83769	0.076
ESCA	0.65235	0.83374	0.1
HNSC	0.00178	0.84992	0.033
KICH	<1e-5	0.98533	<1e-5
KIRC	<1e-5	0.91672	<1e-5
KIRP	<1e-5	0.91075	<1e-5
LIHC	1	0.90053	<1e-5
LUAD	0.05117	0.85152	0.03
LUSC	<1e-5	0.83877	0.066
PRAD	0.00621	0.92561	<1e-5
STAD	0.03263	0.85049	0.032
THCA	<1e-5	0.95903	<1e-5



Oxidative Phosphorylation

Peter Michell

- Question: where do the OH-’s go inside inner membrane of mitochondria?
- Oxidative phosphorylation: The proton gradient drives ATP synthesis

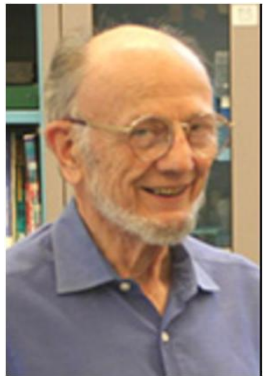


Experimental Validation

- André Jagendorf demonstrated in 1966 that cross-membrane proton gradient can directly drive ATP synthesis by ATP synthase

- … hence experimentally validated Peter Michell's model, and helped Michell to win Nobel Prize in 1978

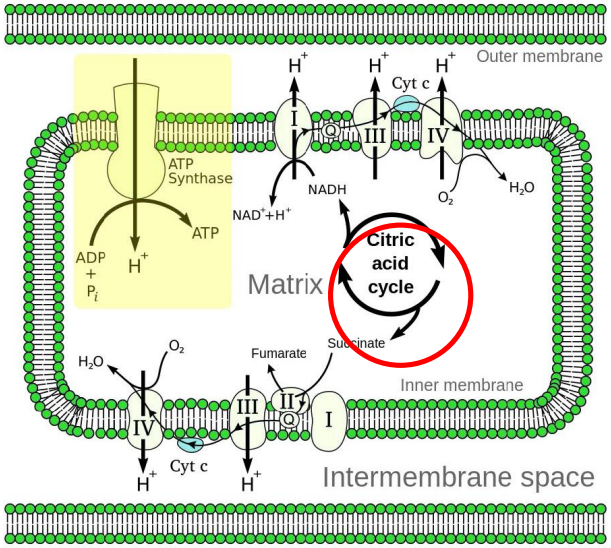
只要水有足够的落差，就能发电

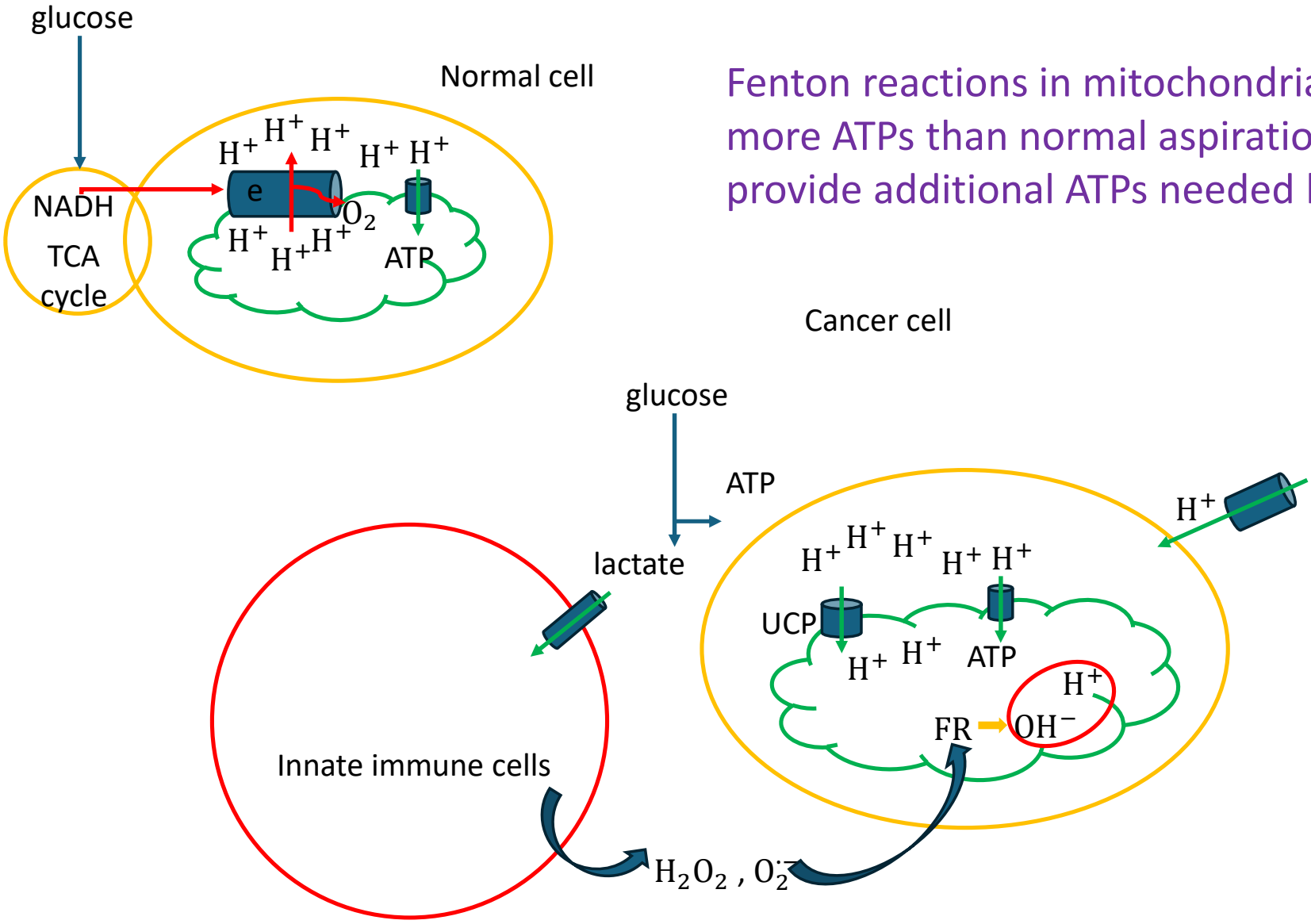


ATP Production by Mitochondria

- It has been established that cancer mitochondria continue to produce ATP through part of the electric transfer chain

- But the TCA cycle is also known to be largely repressed so where the fuel comes from to generate ATP





Fenton reactions in mitochondria generate more ATPs than normal aspiration chain, to provide additional ATPs needed by cancer

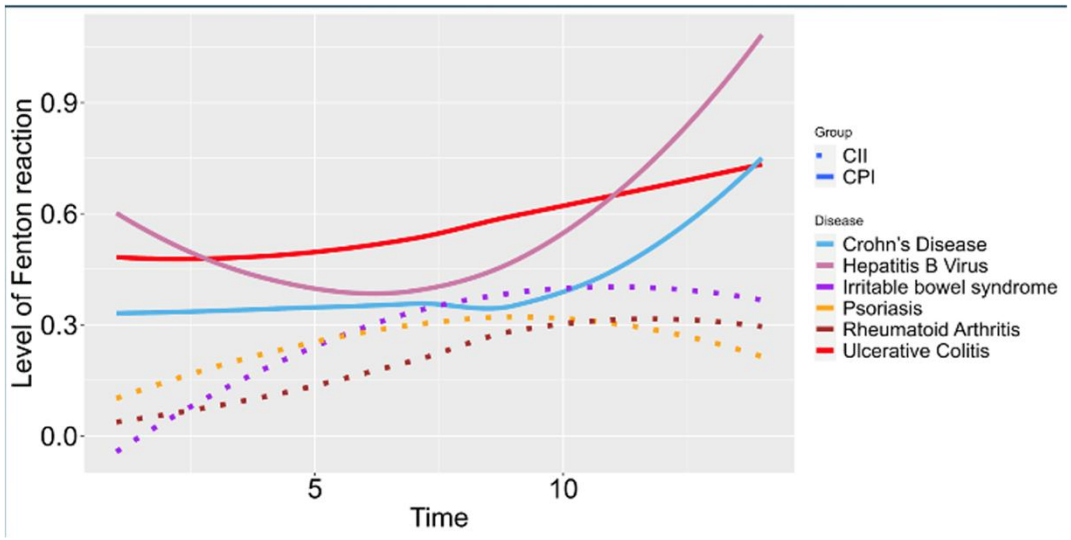
Fenton Reactions in Extracellular Matrix

- All TCGA cancers have Fenton reactions in their extracellular matrix (ECM)
- The hydroxyl radicals produced by persistent Fenton reactions in ECM continuously damage ECM and the tissue structure
- The so damaged tissues attract innate immune cells to the sites and keep inflammation going

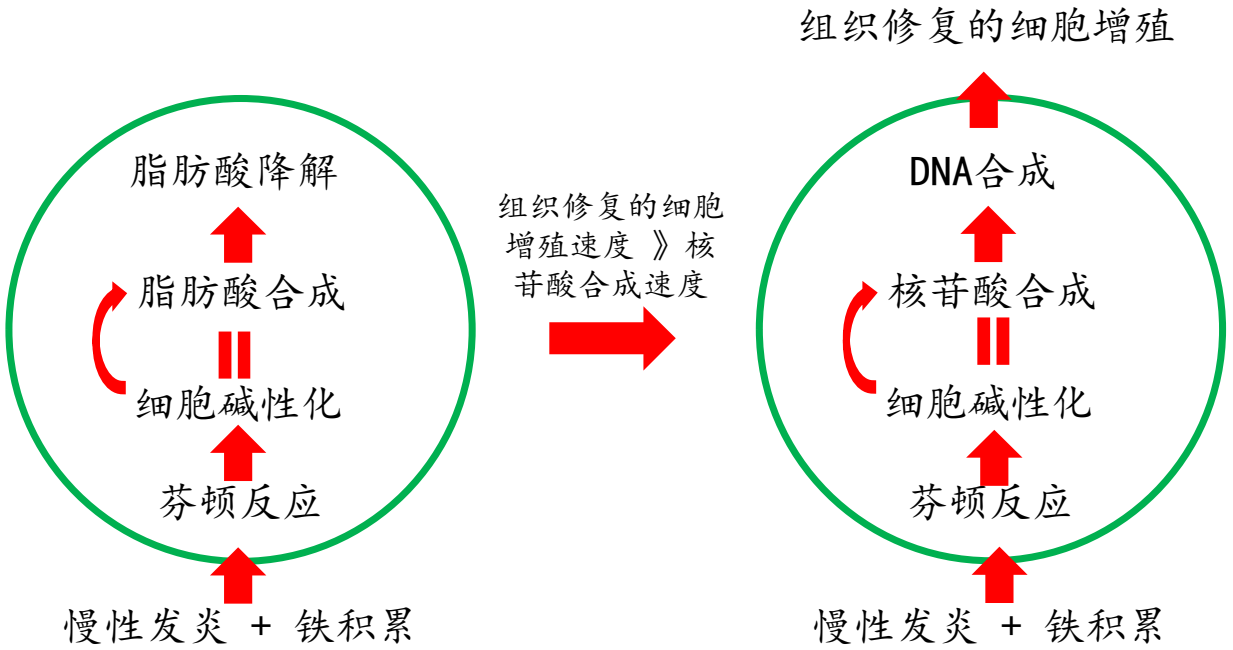
- 如果这是疾病的“稳态”，它是如何演化到这一步的

易癌变和不易癌变的慢性发炎

- 我们分析了十种易癌变及十种不易癌变的慢性发炎疾病组织的转录数据，发现两者的主要差别为：前者的芬顿反应持续增长，而后者很快进入稳态

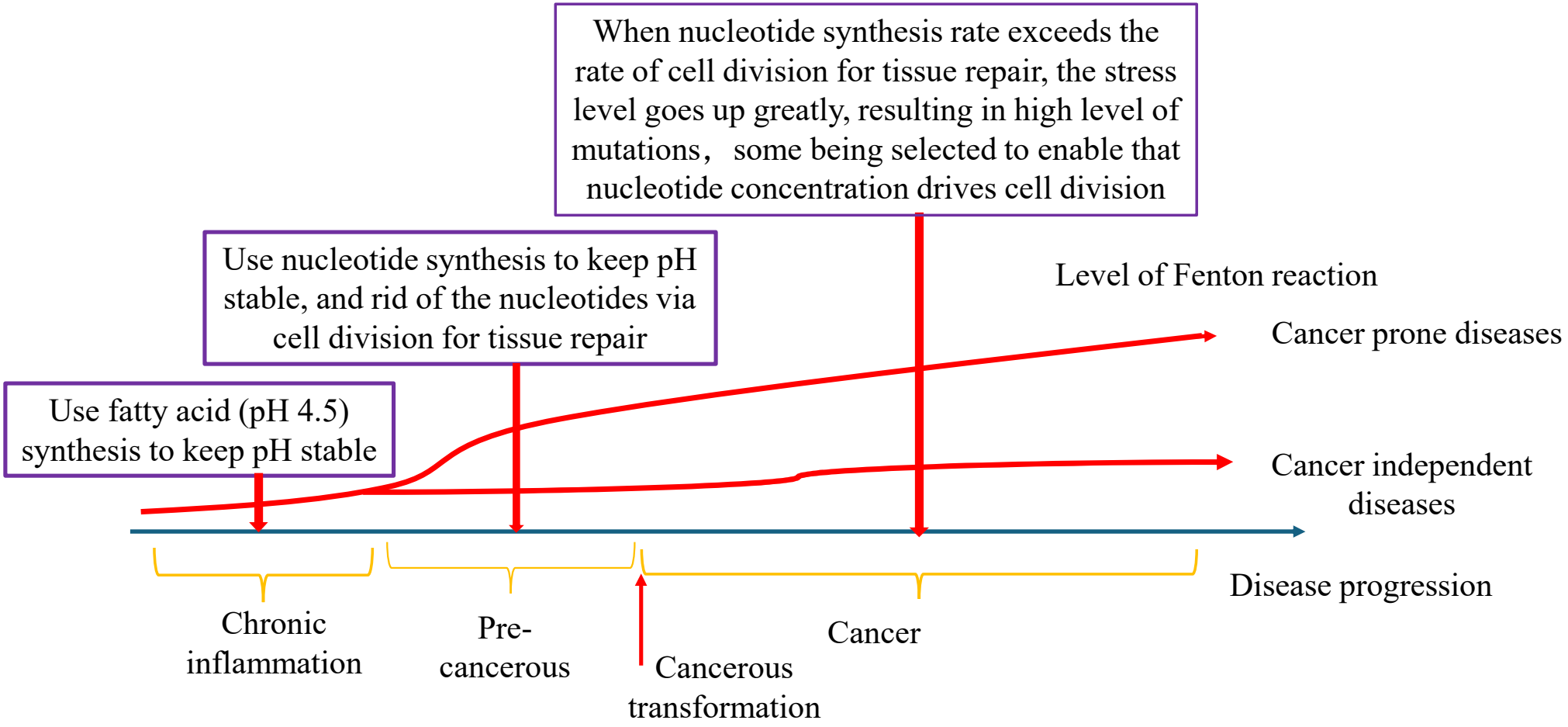


慢性疾病中的芬顿反应及适应性反应



黄振羽

Inflammation-to-cancer Transformation: Finding Metabolic Exits for Nucleotides Synthesized for Survival



Fundamentals to Cancer Research

- Cancer is about survival under persistent alkaline stress
- … through induction of acidifying reprogrammed metabolisms
- The induction of these metabolisms is through
  - epigenomic activity, genomic mutations, and life/death selection – 条条大路通罗马
- So, in addition to the basic metabolic knowledge, we need to know the chemical conditions where the metabolisms are induced

我们的方法学

Systems Engineering from Data to Knowledge: Step 1

- Developing **marker genes for individual** key events and cellular states: Based on omics data, discover and characterize the major stressors, metabolic reprogramming, and aberrant cellular behaviors by diseased tissues and cells, using marker genes for
  - **Stressors**: intracellular alkaline stress, extracellular acidic stress, oxidative stress, hypoxic stress, respectively
  - **Metabolic reprogramming**: the Warburg effect, *de novo* nucleotide synthesis, over-synthesis and utilization of sialic acids, and others.
  - **Aberrant cellular behaviors**: sustained cell proliferation, cell migration, T-cell inactivity

Identification of key individual events

Systems Engineering from Data to Knowledge: Step 2

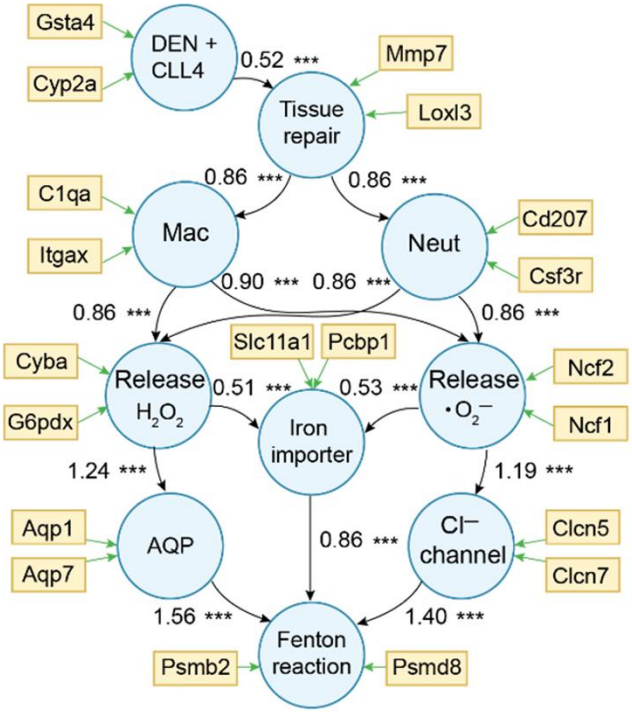
- Example: All RMs conserved across two or more TCGA cancer types produce more H^+ than the corresponding normal metabolic processes (nucleotide *de novo* synthesis vs. salvaging nucleotides from the blood). **Question**: why?
- Through linear regression, we found $\mathbf{R(FR, OH^-) = \sum_i \alpha_i\ R(RM_i, H^+)}$, namely the rate at which all acidifying RMs produce H^+ ($\mathbf{R(RM_i, H^+)}$) is strongly correlated with the rate of OH^- production by Fenton reaction ($\mathbf{R(FR, OH^-)}$)
- By using structural equations, it can be statistically demonstrated that "$\mathbf{R(FR, OH^-)}$" is the cause of each $\mathbf{RM_i}$ – causal analysis

Derive pairwise causal relationships

Systems Engineering from Data to Knowledge: Step 3

- During the inflammation–cancer transformation
  - **Fenton reaction**: converts oxidative stress into cytoplasmic alkaline stress
  - Drives system-level **acidic RMs**, including *de novo* nucleotide synthesis (purine synthesis produces 8–9 H^+)
  - Activate a **somatic hypermutation program** that generates a large number of mutations in the genome
  - Selected mutations **simplify the polarity system** of cancer cells into a yeast-like unicellular polarity system
  - Allow nucleotide concentration to drive cell proliferation – completing the inflammation–cancer transformation

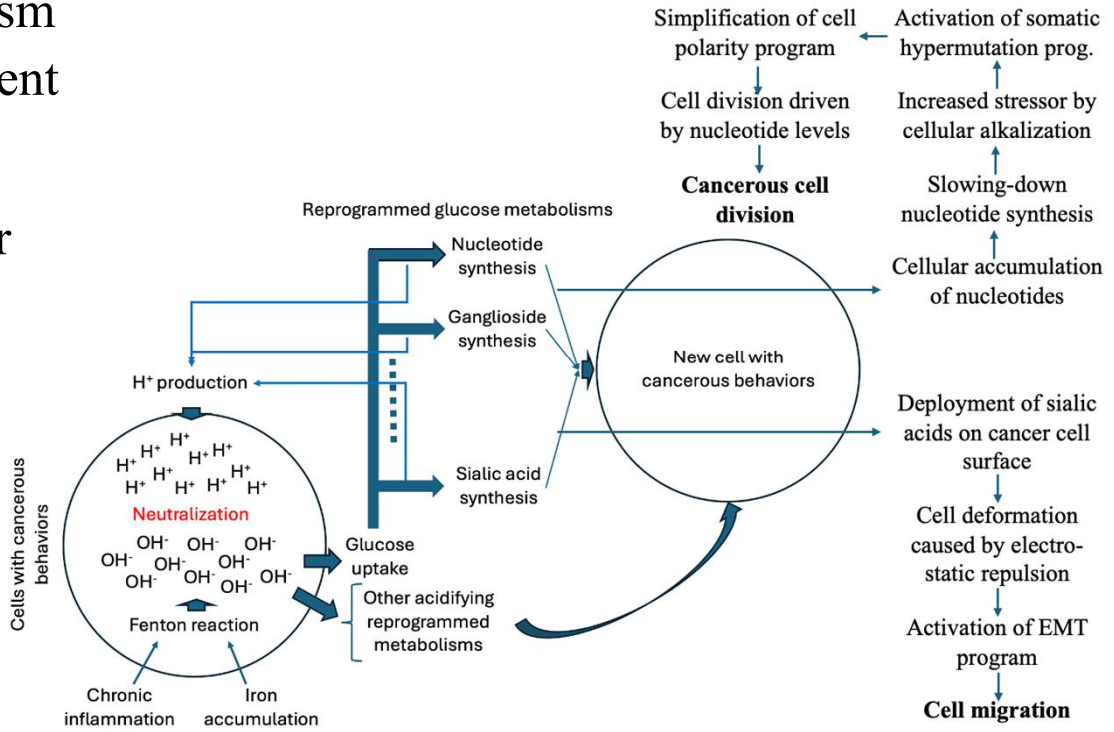
Construct causal networks



Causal diagram of the Fenton reaction induced by DEN in mouse liver disease caused by the chemical carcinogen DEN

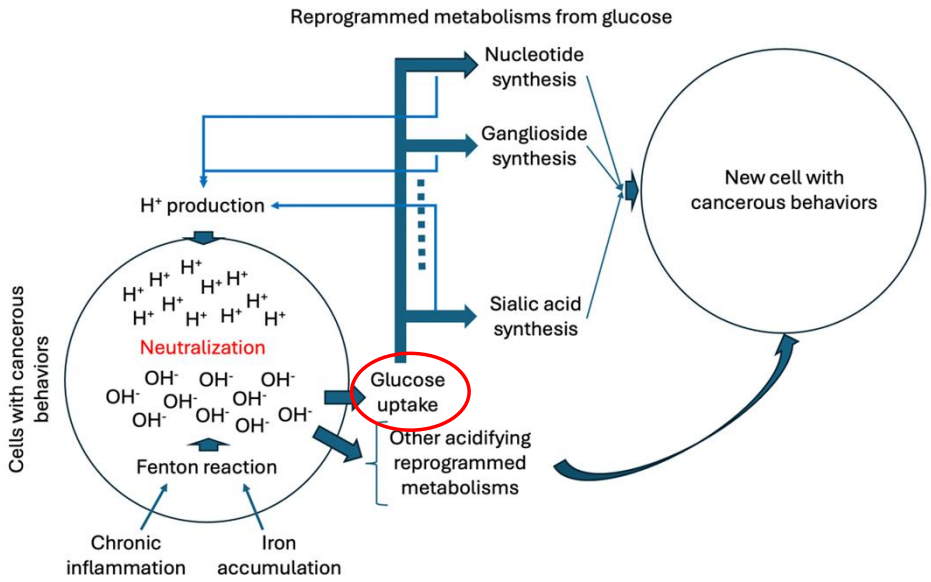
Systems Engineering from Data to Knowledge: Step 4

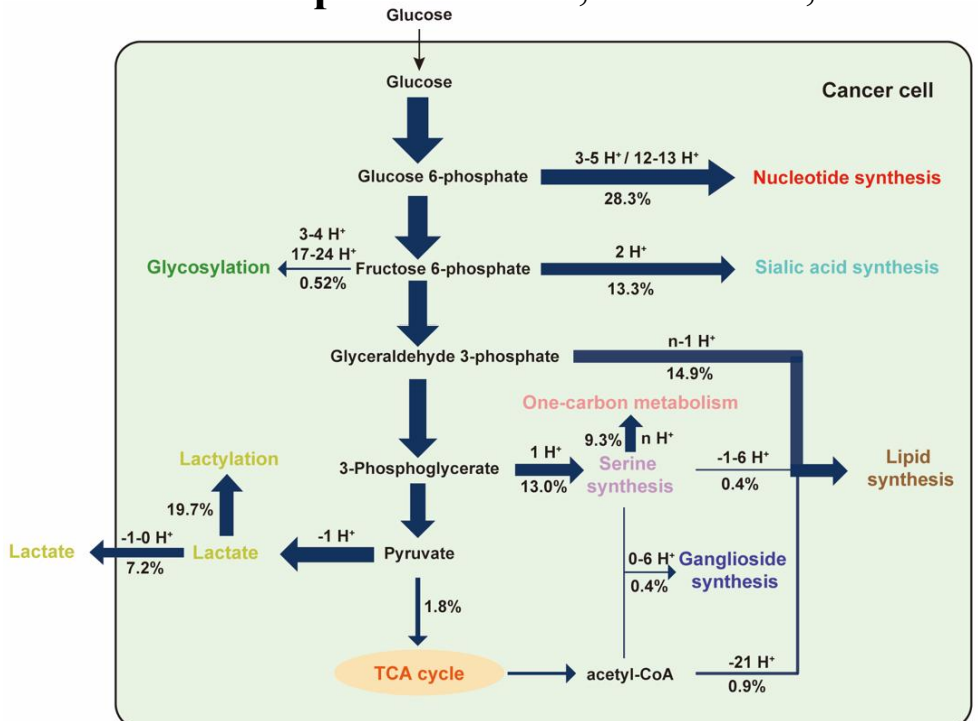
- Using this method, we can construct **a mechanistic model** for the development of a disease
- The figure shows drivers and core mechanism of our predicted model of cancer development
- It can be used to **explain a series of causes and possible mechanisms** related to cancer development
- The model has been validated using mouse liver and ovarian cancer models through collaboration with **Professor Ye Zhang** of China Medical University



Systems Engineering from Data to Knowledge: Step 5

- **Metabolic flux analyses**
  - The acidic RMs in cancer cells mainly **originate from glucose**
  - The **level of glucose flux** into different metabolic branches determines many cellular behaviors: **proliferation rate**, **prone to metastasis** or not, their **relationship with T cells**, redox state, extracellular acidic environment





Summary

- Cancer and cancer-forming cells are under persistent cytosolic alkaline stress
- For survival, the cells utilize acidifying metabolisms to keep the pH stable
- These reprogrammed metabolisms dictate the behaviors of the host cells
- In addition to cytosolic alkaline stressor, cancer cells also face other less-threatening stressors such as extracellular acidity, hypoxia, oxidative stress and competition for limited nutrients, which may drive some metabolic reprogramming